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Intravenous and oral clozapine pharmacokinetics, pharmacodynamics, and concentration–effect relations: acute tolerance

Lei Sun [a], Chyan E. Lau [b,*]

[a] *Department of Chemistry, Rutgers, The State University of New Jersey (L.S.), 152 Frelinghuysen Road, Piscataway, NJ 08854-8020, USA*
[b] *Department of Psychology, Rutgers, The State University of New Jersey (C.E.L.), 152 Frelinghuysen Road, Piscataway, NJ 08854-8020, USA*

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Abstract

We examined the pharmacokinetics and pharmacodynamics of intravenous (1–5 mg/kg) and oral clozapine (2.5–10 mg/kg) in rats (terminal half-life = 81.8 min; oral bioavailability = 5.32%). Both dose– and concentration–effect relations of clozapine were characterized. Clozapine's effects were similar to those of benzodiazepines because of the similarity in effect–time profiles between the two classes of drugs. The IC_{50} value increased as a function of dose; consequently, clozapine's relative potency decreased linearly with the logarithm of $AUC_{(0-\infty)}$, or bioavailable dose regardless of route of administration. The IC_{50} is an index for the sensitivity of behavioral performance to clozapine; relative potency provides an index for estimating the extent of acute tolerance. As IC_{50} increases, relative potency decreases, and consequently, acute tolerance increases. Our results demonstrated that greater acute tolerance was observed for i.v. clozapine than for p.o. clozapine; however, clozapine exhibited a single concentration–effect relation across dose and route of administration after correcting for relative potencies.

Keywords: Acute tolerance; Clozapine, intravenous; Clozapine, oral; Pharmacodynamics; Pharmacokinetics; Pharmacokinetic–pharmacodynamic modeling; Potency, relative

1. Introduction

Clozapine is an atypical antipsychotic agent with proven efficacy in the management of refractory schizophrenia (Stille et al., 1971; Fitton and Heel, 1990). Pharmacologically, clozapine is a potent antagonist of dopamine and a number of other receptors, e.g., 5-HT (Richelson, 1984; Meltzer et al., 1989). Its effects have been extensively studied in animal research using various behavioral paradigms (Canon and Lippa, 1977; Canon, 1979; Wenger, 1979; Schaefer and Michael, 1980; Spealman and Katz, 1980; Spealman et al., 1983; Sanger, 1985; Seiden et al., 1985; Fowler and Liou, 1998). Despite its clinical use as a neuroleptic agent, clozapine's behavioral profiles differ notably from those of typical neuroleptic agents in animals (e.g., Canon and Lippa, 1977; Canon, 1979; Schaefer and Michael, 1980; Spealman and Katz, 1980; Spealman et al., 1983) but are similar to those of anxiolytics like benzodiazepines (Spealman and Katz, 1980; Spealman et al., 1983). Moreover, like benzodiazepines, clozapine exhibits dose-related, biphasic effects (Canon, 1979); it increases response rates at lower doses and decreases rates of responding at higher doses. Unlike typical neuroleptics (e.g., haloperidol), clozapine produces a low incidence of extrapyramidal side effects (De Maio, 1972).

In past research, we integrated pharmacokinetics and pharmacodynamics to study the biphasic effects of benzodiazepines (e.g., alprazolam) under an acute dose regimen (Lau and Heatherington, 1997; Lau et al., 1998). The pharmacodynamic measure used in those studies was performance under the differential reinforcement of low rate 45-s schedule, which produces ''spaced responding'' or ''timing'' behavior. The differential reinforcement of low rate 45-s schedule of reinforcement results in low rates of responding, as only those responses that occur after a minimum time interval (in this case, 45 s) following a previous response are reinforced; responses that occur before 45 s have elapsed are not reinforced, and the timing interval is reset. Inter-response time profiles and the num-

* Corresponding author. Tel.: +1-732-445-2543; fax: +1-732-445-5147.
E-mail address: clau@rci.rutgers.edu (C.E. Lau).

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ber of responses can be recorded throughout the session. That drugs can alter the inter-response time distribution and disturb its sequential patterning permits one to characterize their putative actions from their effect–time profiles. The density of reinforcement and shorter-response (or non-reinforced) rate were used to study dose–response and effect–time profiles of drugs in our previous pharmacokinetic–pharmacodynamic studies cited in this paper.

Although effects of clozapine on differential reinforcement of low rate performance have been investigated in animals, analyses were limited to time-course data collapsed into single points for constructing dose–response relations rather than effect–time profiles (Canon, 1979; Seiden et al., 1985). We first aim to characterize clozapine's effects on the two pharmacodynamic measures under the differential reinforcement of low rate 45-s schedule for i.v. and p.o. clozapine in rats. Analyzing the dose–response relations and the effect–time profiles in the same way as those for alprazolam and midazolam (Lau et al., 1997, 1998) provides comparison between clozapine's behavioral profiles and those of the two benzodiazepines, because pharmacodynamically, clozapine more closely resembles those benzodiazepines than typical neuroleptics (e.g., Spealman and Katz, 1980). The dose–response curves also allow comparison of overall potency and efficacy between intravenous and oral clozapine, whereas effect–time profiles reflect their continuous pharmacokinetic profiles and describe distinct ongoing pharmacological effects.

In humans, oral clozapine is rapidly absorbed and extensively metabolized with a terminal half-life of approximately 6 h, and exhibits linear kinetics in the therapeutic range (Jann et al., 1993; Dain et al., 1997). Estimated bioavailability of orally administered clozapine ranges from 27% to 50% (Choc et al., 1990; Fitton and Heel, 1990). Effects of clozapine have previously been characterized in animals only using dose–effect relations by extravascular routes of administration (Canon, 1979; Seiden et al., 1985), but not concentration–effect relations. The second aim then, was to characterize the pharmacokinetics of i.v. and p.o. clozapine. The i.v. route was chosen for its rapid onset of action without interference from absorption and first-pass effect, enabling assessment of the absolute bioavailability of oral clozapine. This pharmacokinetic investigation was to be conducted in animals of the same species, age, gender, and under the same food-limited regimen used in the pharmacodynamic study.

The serum drug concentration–effect relations provide a more accurate indication of drug action than dose administered–effect relations, because pharmacodynamic parameters are estimated using serum concentrations rather than dose. This is important since pharmacokinetics typically differs widely between drugs and even among different routes of administration. For example, a p.o. 20 mg/kg cocaine dose was shown much more effective than an i.v. 2 mg/kg dose in disrupting differential reinforcement of low rate performance (Ma et al., 1999). Furthermore, the integration of pharmacokinetics and pharmacodynamics permits partition of pharmacokinetic and pharmacodynamic components in drug action. Thus, a third aim of integrating pharmacodynamics with the corresponding pharmacokinetics for both i.v. and p.o. clozapine was to identify, and thereby quantify, any emerging events in the time course of drug action, such as acute tolerance. Simultaneous pharmacokinetic–pharmacodynamic analysis enables the investigation of a single concentration–effect relation for clozapine regardless of the dose or route of administration used.

2. Materials and methods

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

2.1.1. Animals

Four male, adult, albino rats of the Sprague–Dawley strain from HSD (Indianapolis, IN) with a mean, initial body weight of 384 g (range: 380–388 g) were used. Body weights were reduced to 80% of free-feeding levels by limiting daily food rations over a 2-week period as described previously (Lau et al., 1999a), and held at this for the duration of the experiment. They were housed individually in a temperature-regulated room with a 12 h light–dark cycle (lights on at 0700 h). Water was continuously available in the living cages. Experiments were conducted 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

Clozapine (obtained from Sandoz Pharmaceuticals [E. Hanover, NJ, USA]) was prepared by dissolving 5 mg of the drug in 25 μl of 1.2 N HCl, then further diluting it to working concentration with 0.9% NaCl solution. Clozapine was administered in a volume of 1 ml/kg body weight either intravenously or orally by gavage. When an i.v. bolus dose of clozapine was administered, clozapine solution was delivered in 30 s and was followed by 1 ml 0.9% saline in 15 s.

2.1.3. Catheterization

Animals were weighed prior to surgery and then anesthetized with ketamine and xylazine as described previously (Lau et al., 1996; Ma et al., 1999). Right jugular vein cannulation was performed under sterile conditions. When not in use, the catheter was flushed with 0.9% saline containing 50 units of heparin per milliliter and was sealed with fishing line.

2.1.4. Apparatus

Four experimental Plexiglas chambers were used, and have been described previously (Lau and Wang, 1996).

Each chamber, enclosed in a sound-attenuating shell, was equipped with a response lever and stainless steel food-pellet receptacle into which 45-mg dustless pellets (BioServ, Frenchtown, NJ) could be delivered. Session contingencies were programmed and data were recorded using QuickBasic on an IBM-type 486 X computer.

2.1.5. Procedure

Rats were trained to respond under a differential reinforcement of low rate 45-s schedule as described previously (Lau and Heatherington, 1997). Once training was complete, a 3-h session was conducted at the same time each day. After the intersession performance had stabilized (i.e., the total and reinforced responses did not vary by more than 5% from the baseline for 5 consecutive days), right jugular vein catheters were implanted as described above.

The animals were allowed at least 2 days to recover from jugular vein catheterization before being returned to the daily operant sessions. Once intersession performance had reestabilized, drug administration series began. All injections were given immediately prior to a session, and were separated by 3–5 days in random order within a series. We found that a preceding dose has no residual effect on the succeeding dose (e.g., tolerance, sensitization) for drugs such as cocaine and caffeine when within-drug doses were separated by 3–5 days (Wang and Lau, 1998; Lau et al., 1999a). The animals first received i.v. clozapine with administration of vehicle, 1, 2.5 and 5 mg/kg. All these clozapine bolus doses were administered in 30 s, with resulting rates of input for the i.v. 1, 2.5 and 5 mg/kg doses at 0.011, 0.027 and 0.053 mg/s, respectively. The second dosing series consisted of an oral clozapine dose–response determination (vehicle, 2.5, 5 and 10 mg/kg). Each dosing series was separated by 10 non-injection sessions.

2.1.6. Data analyses

The inter-response time distributions after the administration of vehicle and clozapine doses were analyzed for 3-h sessions. The first 2 min of data, which allowed for the transient effects of handling, were not included in the analysis. Baseline inter-response time distributions for each session immediately preceding an injection also were analyzed. For each rat, there were four baseline-day values that were averaged and treated as the mean baseline effect for each route of administration. Responses with inter-response times ≥ 45 s (reinforced) and those with inter-response times < 45 s (shorter or non-reinforced) were extracted from the inter-response time distributions and were expressed as rate (responses per minute). The total number of responses consisted of responses with inter-response times ≥ 45 and < 45 s. Efficiency was calculated as the ratio of reinforced responses to total responses. The four parameters were transformed to mean percent baseline values to compensate for individual differences in performance; that is, the effect is expressed as a function of baseline ($E = E_c/E_b$), where E denotes effect, E_b baseline effect, and E_c clozapine effect.

Although we found that the density of reinforcement in both the 45–55 and ≥ 45 s bins decreased as a function of dosage for drugs (e.g., alprazolam, caffeine), the 45–55 s bin function was more sensitive to drug effects than was the total density of reinforcement (≥ 45 s). The 45–55 s bin function also required lower doses to reach maximum effect than did the measure of the total density of reinforcement, and it has been used previously to characterize the relation between pharmacokinetics and pharmacodynamics following drug administration under an acute dose regimen. Thus, in the present study, we also analyzed the inter-response times in the 45–55 s bin to facilitate a comparison with our previous studies.

Dose–response curves for intravenous and oral clozapine were constructed using a four-parameter, logistic function of the following equation by the ALLFIT curve-fitting program written for the IBM PC (DeLean et al., 1992):

$$y = \left[(a-d) / \left(1 + (x/c)^{b}\right)\right] + d$$

where, y is the percent of baseline performance in the 45–55 s bin and x is the drug dose administered. The four fitted parameters were: a, the E_{min}, i.e., the % baseline performance when $x = 0$; d, the E_{max}, i.e., the % baseline performance for ''infinite'' dose; b, the slope factor which determines the steepness of the curve; c, the ED_{50}, i.e., the dose resulting in a response halfway between a and d.

Regression analysis and repeated-measures, one-way or two-way analysis of variance (ANOVA), followed by Newman–Keuls tests using SigmaStat (Jandel, San Rafael, CA) for the evaluation of clozapine's effects, were performed as appropriate.

2.2. Pharmacokinetics

2.2.1. Animals

Seven male rats of the same strain were placed under conditions, including a food-limited regimen, similar to those used in the pharmacodynamic study. They were reduced to 80% of their initial, adult free-feeding body weights (mean = 384 g; range: 380–393 g) as described above and held at this weight for 3 months before the start of the experiment, the time period needed for training and establishing behavioral baseline performance.

2.2.2. Reagents and high-performance liquid chromatography (HPLC)

α-Hydroxymidazolam was obtained from Hoffmann–LaRoache (Nutley, NJ, USA). *N*-Desmethylclozapine was purchased from Sigma (St. Louis, MO). Reagents were obtained from standard commercial sources. A rapid and sensitive HPLC microsample (50 μl) method for the determination of clozapine and *N*-desmethylclozapine has been

described previously (Ma and Lau, 1998). The separation of clozapine, *N*-desmethylclozapine and α-hydroxymidazolam (as an internal standard) was performed on a Symmetry C_{18} column, 150 × 2.1 mm I.D., 5 μm particle size (Waters Associates, Milford, MA). We used an isocratic mobile phase consisting of methanol–acetonitrile–28.6 mM sodium acetate buffer pH 2.6 (10:20:70, v/v/v). Clozapine and *N*-desmethylclozapine were separated from serum samples by liquid–liquid extraction. The detection limit was 2.5 ng/ml for each agent using a UV detector at 230 nm. The within-day and between-day precisions for clozapine were high with the coefficients of variation in the range of 2.69–7.31% and 3.02–7.58%, respectively.

2.2.3. Clozapine administration and blood sampling

The animals were allowed to recover for at least 2 days from jugular vein catheterization prior to the drug administration series. Animals were divided into two groups. Group 1 ($N = 4$) received i.v. bolus doses of clozapine (1, 2.5 and 5 mg/kg) via the jugular vein catheter. Group 2 ($N = 3$) received an i.v. bolus dose (2.5 mg/kg) and a p.o dose (10 mg/kg) of clozapine. Each drug dose was separated by 3–5 days in random order. The blood sampling procedure has been described previously (Ma et al., 1999). Blood samples (100 μl) were obtained following i.v. 1 mg/kg clozapine administration at 5, 10, 15, 20, 30, 45, 60 and 90 min post-injection. For the i.v. 2.5 and p.o. 10 mg/kg doses, blood samples were also obtained at 120 min. An additional blood sample was obtained at 180 min for the i.v. 5 mg/kg dose. To minimize the difference between the pharmacokinetic and pharmacodynamic animals, the animal received 45-mg food pellets matched in number with those earned during the corresponding differential reinforcement of low rate 45-s sessions at time points of 15, 30, 45, 60, 90, 120 and 180 min for each clozapine dose. After 180 min, the animals were returned to home cages and given food rations sufficient to maintain their usual, daily criterion weights.

2.3. Pharmacokinetic–pharmacodynamic modeling

We used the SAAM II software system (SAAM Institute, 1997) to perform pharmacokinetic–pharmacodynamic analyses. To prevent any effect of blood sampling on behavioral performance, the pharmacokinetic and pharmacodynamic studies of clozapine were conducted in a between-subject design as performed in our previous studies (Lau and Heatherington, 1997; Lau et al., 1997, 1998, 1999a) using pooled data for the pharmacokinetic–pharmacodynamic analyses, as was done with a cocaine study (Lau et al., 1999a). For example, in the i.v. dosing group, pooled data consisted of the pharmacokinetic and pharmacodynamic data sets for all three doses from each animal; that is, 104 concentrations for the pharmacokinetic data and 120 observations for the density of reinforcement measure. Assessment of the goodness of fit of each proposed model to experimental data was based on Akaike's information criterion (AIC) to evaluate model order and to perform model discrimination.

2.3.1. Pharmacokinetic analysis

Serum concentration–time profiles were analyzed using compartmental modeling. For Group 1 (s1–s4), the clozapine serum concentration–time profile was modeled with an open two-compartment (cpt) system with elimination from the central cpt following i.v. administration. Three pharmacokinetic models were used (Fig. 1, central panel), one for each dosing regimen. Each model contained the same set of pharmacokinetic parameters for the four animals: the volume of distribution at the central cpt (V_c) and the rate constants [$k_{(2,1)}$, $k_{(1,2)}$ and $k_{(0,1)}$]. These pharmacokinetic parameters were estimated by simultaneously fitting all data and used to calculate distribution and elimination rate constants, α and β, respectively, by standard formulae. The area under the serum clozapine concentration–time curve from time 0 to infinity [$AUC_{(0-\infty)}$] was obtained from SAAM II software. The volume of distribution at steady state (V_{ss}), total clearance (C1), and the mean residence time (MRT) for i.v. clozapine were calculated by standard noncompartmental analysis.

For Group 2 (s5–s7), a two-cpt model with an added absorption cpt was used to analyze the clozapine's serum concentration–time profile after p.o. 10 mg/kg administration (Fig. 1, central panel). k_a is the first-order absorption rate constant for clozapine from the absorption site. The absolute bioavailability (F) is the fraction of oral clozapine dose entering the central cpt intact as clozapine. We then analyzed the i.v. 2.5 and p.o. 10 mg/kg clozapine concentration–time profiles simultaneously, assuming the

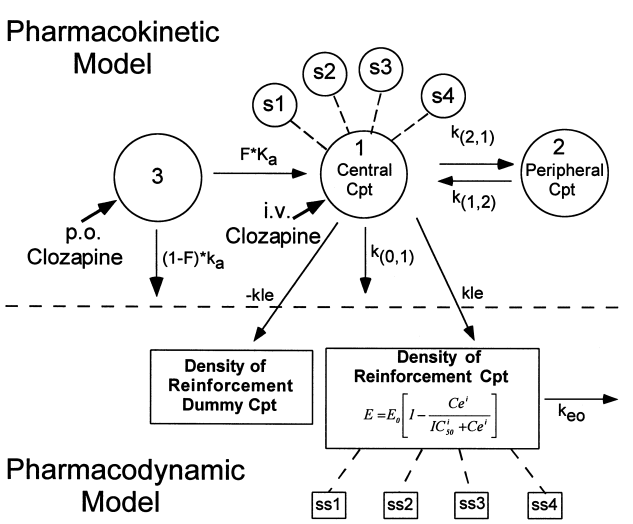

Fig. 1. Diagrammatic representation of integrated pharmacokinetic–pharmacodynamic Models 1 or 2 [Pharmacokinetics: Group 1 ($N = 4$; s1–s4, central panel); Group 2: ($N = 3$; s5–s7, not shown). Pharmacodynamics: ($N = 4$; ss1–ss4)] used to describe the density of reinforcement (lower panel) after administration of a single i.v. or p.o. dose of clozapine.

distribution and elimination characteristics were the same, except for the absorption phase regardless of the route of administration.

2.3.2. Pharmacodynamic model

The density of reinforcement (45–55 s bin) was used as a behavioral endpoint to analyze concentration–effect relations of clozapine. A multi-compartmental model incorporating one link cpt, representing density of reinforcement cpt, was used to describe the data and has been described previously (Lau et al., 1999a). This effect–link model was based on one proposed by Sheiner et al. (1979), wherein an effect cpt is linked to the central cpt via the first-order rate constant ($k1e$), which is very small relative to the other rate constants (Fig. 1). The parameter $k1e$ was fixed at 0.0001 min^{-1}, a numeric value that has been shown to be of no consequence (Sheiner et al., 1979). Drug effect–site kinetics was defined by the loss rate constant, k_{eo}. Clozapine decreased the density of reinforcement from baseline (100%) to 0% (e.g., Fig. 4C–D); thus, the inhibitory E_{max} model was used to describe and predict this pharmacodynamic measure, which is expressed in terms of Ce such that

$$E = E_0\left[1 - \frac{Ce^i}{Ce^i + IC_{50}^i}\right],$$

where E_0, IC_{50} and Ce are the baseline response, the clozapine concentration required to produce 50% maximal inhibition, and the concentration in the density of reinforcement cpt, respectively, and i is the Hill factor.

2.3.3. Integration of pharmacokinetics and pharmacodynamics

Once initial pharmacokinetic parameter estimates were obtained for the two groups as described above, two integrated pharmacokinetic–pharmacodynamic models were generated (Models 1 and 2). For example, for Model 1, three pharmacokinetic models (one for each i.v. dose) were integrated with their corresponding pharmacodynamic models (Fig. 1, lower panel). All data were fitted simultaneously within a group; we used the principle of parsimony to examine whether parameters could be shared. Only parameters resulting from Models 1 (Group 1) and 2 (Group 2) were presented. Fig. 1 shows a diagrammatic representation of the pharmacokinetic–pharmacodynamic models for a clozapine dose.

3. Results

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

Fig. 2 shows the effects of i.v. and p.o. clozapine on inter-response time distributions for the 3-h sessions.

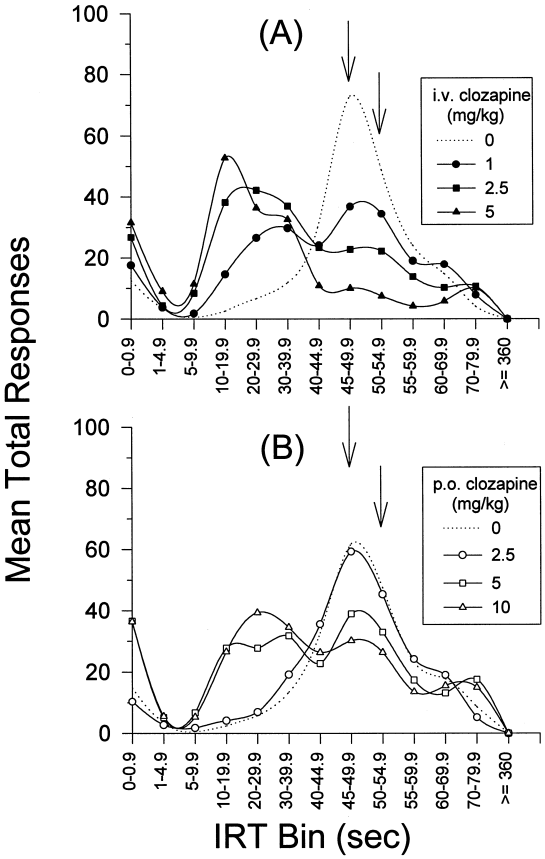

Fig. 2. Mean effects of clozapine on inter-response time distributions during the 3-h sessions: (A) i.v. clozapine (0–5 mg/kg); (B) p.o. clozapine (0–10 mg/kg). All responses before the first arrow are non-reinforced (< 45 s); others are reinforced (≥ 45 s). The region between the two arrows is the 45–55 s bin ($N = 4$). Responses between the two arrows (45–55 s bin) were used for the reinforced-response analysis. To clarify responding at the short end of the inter-response time distribution, burst responses (< 1 s) are shown, and then, the short inter-response times (the next two bins). Adjacent 5-s bins around the criterion bin also are shown.

Clozapine increased the shorter inter-response times (< 45 s) in a dose-related fashion regardless of route of administration. Clozapine decreased the inter-response times in bins ≥ 45 s as a function of dose, with the major decreases detected in bins 45–54.9 s. However, clozapine increased longer inter-response times in the bin of 70–79.9 s somewhat in comparison to those for the vehicle administration.

Fig. 3 shows an overview of performance for the 3-h sessions following intravenous and oral clozapine administration. Clozapine significantly decreased the density of reinforcement in the bins of 45–55 and ≥ 45 s in a dose-related fashion, regardless of route of administration ($P < 0.005$) as reflected by repeated-measures one-way ANOVAs (Fig. 3A). The dose–response curve for the effects of i.v. clozapine on the bins of 45–55 s was parallel to and significantly smaller than that for the bins of ≥ 45 s by a two-way ANOVA ($P < 0.05$); however, no significant difference was found between the two dose–response

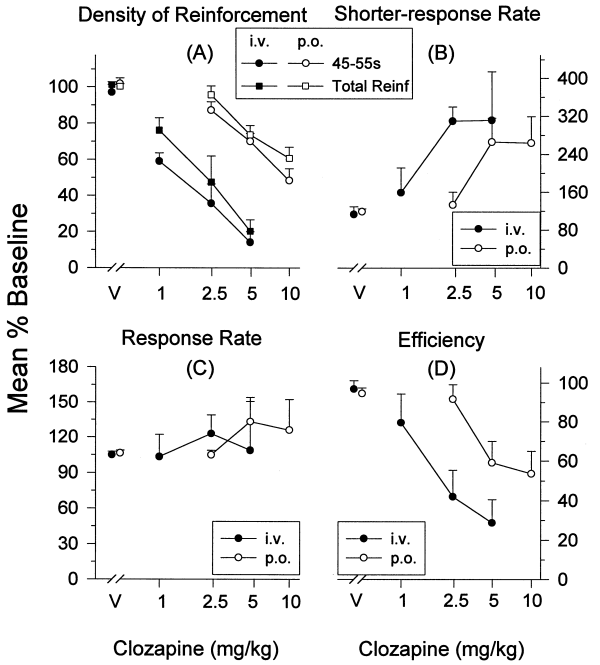

Fig. 3. Mean (S.E.M.) % baseline dose–response curves after i.v. clozapine (0–5 mg/kg) and p.o. clozapine (0–10 mg/kg) administration for the 3-h sessions: (A) density of reinforcement in the > 45 and 45–55 s bins; (B) shorter-response rate; (C) total response rate; (D) efficiency. The abbreviation V denotes vehicle.

curves for the p.o. doses ($P = 0.115$). Hereinafter, the term density of reinforcement refers to the density of reinforcement in the 45–55 s bin. Although both i.v. and p.o. clozapine produced effects on the shorter-response rate, the overall increases were not statistically significant, $P > 0.05$ (Fig. 3B); however, the post hoc Newman–Keuls comparison revealed that the increases in shorter-response rate for the i.v. 1 and 2.5 mg/kg doses were statistically significant ($P < 0.01$). The opposing relation between the density of reinforcement and the shorter-response rate after clozapine administration resulted in a somewhat flat total response rate for i.v. and p.o. clozapine ($P > 0.5$; Fig. 3C). Efficiency for clozapine via the two routes of administration decreased as a function of dose ($P < 0.05$; Fig. 3D). The i.v. and p.o. vehicle injections produced negligible effects, with values for the four performance indices remaining at baseline ($P > 0.05$).

We analyzed the dose–response curves of the density of reinforcement simultaneously after i.v. and p.o. clozapine administration for each animal. Inasmuch as the dose–response curves for i.v. and p.o. clozapine were parallel as shown in Fig. 3A, three parameters of the logistic function (i.e., E_{min}, E_{max} and slope) were shared for the two curves. Because E_{max} had not been reached with either the i.v. or p.o. clozapine doses used, E_{max} was fixed at 0% on the basis of effect–time profiles (see below, e.g., Fig. 4C). The values of E_{min} and slope were 112.74% ($\pm$2.38) and 1.24 ($\pm$0.25), respectively, whereas ED_{50} for i.v. and p.o. clozapine were 1.41 ($\pm$0.10) and 9.67 ($\pm$0.64) mg/kg, respectively. The relative potency measured by the ratio of the two ED_{50} was 6.86 (i.e., 9.67/1.41), indicating that i.v. clozapine was 6.86 times more potent in decreasing the density of reinforcement than was oral clozapine.

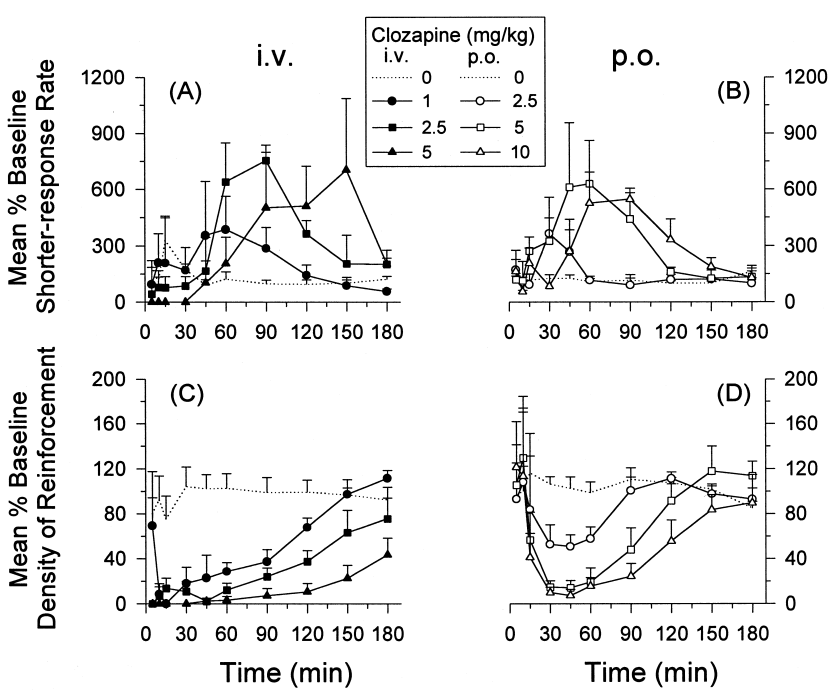

Fig. 4. Effects of i.v. (0–5 mg/kg) and p.o. (0–10 mg/kg) clozapine on effect–time profiles, expressed as % baseline (S.E.M.): (A) and (B) shorter-response rate; (C) and (D) density of reinforcement.

The effects of vehicle via the two routes of administration on the shorter-response rate–time profiles and the density of reinforcement–time profiles were close to baseline with some minor variations (Fig. 4A–D). After i.v. clozapine administration (Fig. 4A), the shorter-response rate increased to maximal effect in a time-related fashion for the three doses ($P < 0.0001$) with the peak effects occurring at 60, 90 and 150 min for the 1, 2.5 and 5 mg/kg doses, respectively. However, the increase in shorter-response rate across doses was not statistically significant in terms of magnitude ($P > 0.5$). Likewise, oral clozapine increased the shorter-response rate as a function of time with the peak effects occurring at 30, 45 and 90 min for the 2.5, 5 and 10 mg/kg doses, respectively, $P < 0.005$ (Fig. 4B), although the increase across doses was not statistically significant ($P > 0.5$). In contrast to its effect on the shorter-response rate, i.v. clozapine decreased the density of reinforcement in a dose- and time-related fashion ($P < 0.005$ and $P < 0.000005$, respectively) as reflected by repeated-measures two-way ANOVAs (Fig. 4C). The maximal effect of intravenous clozapine for all doses occurred immediately except for the lowest dose (1 mg/kg). The density of reinforcement returned toward or to baseline in a dose- and time-related fashion; however, it remained low at the end of the session for the highest dose (5 mg/kg). Likewise, effects of oral clozapine on the density of reinforcement exhibited a dose- and time-dependent relation ($P < 0.05$ and $P < 0.01$, respectively; Fig. 4D) with the maximal effect occurring at 45 min instead of immediately, as it did for i.v. clozapine.

3.2. The pharmacokinetics of clozapine

3.2.1. Group 1: intravenous 1–5 mg / kg clozapine

The serum concentration–time profiles of clozapine and pharmacokinetic parameters for i.v. clozapine (1–5 mg/kg) are shown in Fig. 5A and Table 1 (left panel), respectively. Clozapine was eliminated according to a biphasic process with a distribution half-life ($t_{1/2}\alpha$) of 6.89 min and a terminal elimination half-life ($t_{1/2}\beta$) of 81.8 min. Dose dependency was not observed. Clozapine $AUC_{(0-\infty)}$ increased linearly as a function of dose (Table 1). Fig. 5B, as

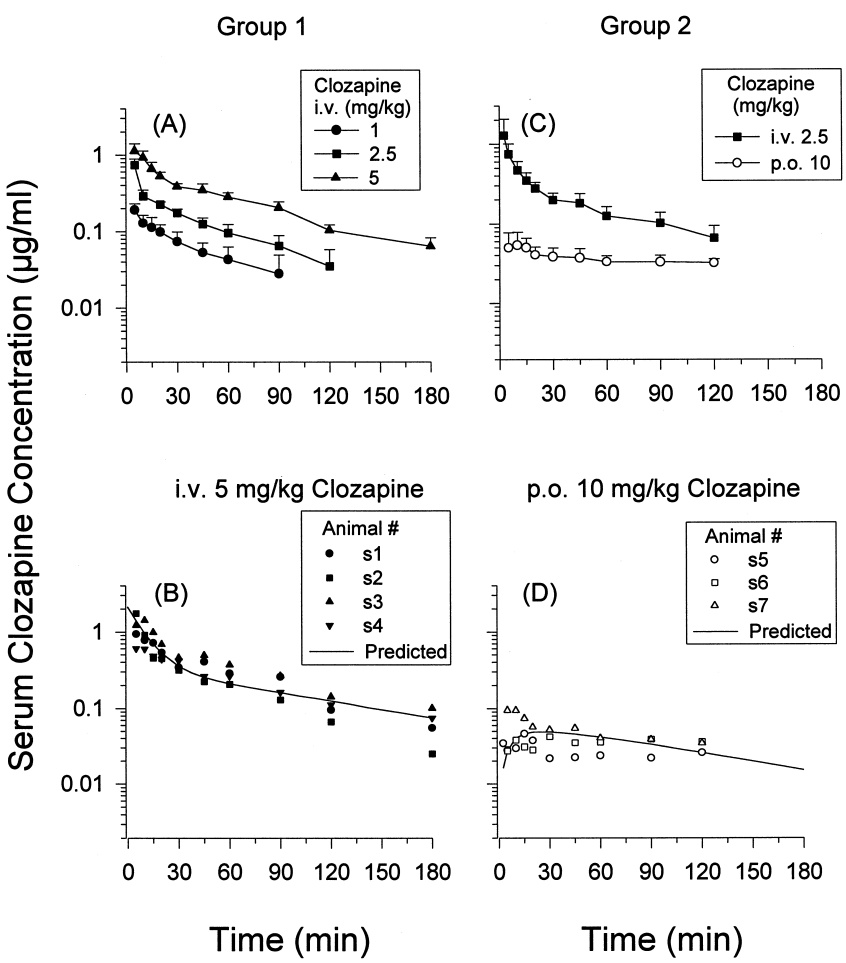

Fig. 5. Serum clozapine concentration–time profiles for Group 1 after: (A) i.v. clozapine administration [1–5 mg/kg; mean (S.E.M.)]; (B) 5 mg/kg [individual data (s1–s4) and predicted profile]. Serum clozapine concentration–time profiles for Group 2 after: (C) i.v. 2.5 and p.o. 10 mg/kg administration [mean (S.E.M.)]; (D) 10 mg/kg [individual data (s5–s7) and predicted profile].

Table 1
Clozapine pharmacokinetic and pharmacodynamic parameters (CV%) estimated by simultaneous pharmacokinetic–pharmacodynamic modeling of serum clozapine concentration–time profiles and density of reinforcement–time profiles after administration of i.v. bolus clozapine (1–5 mg/kg)
The superscript ''*x*'' denotes the respective clozapine doses (1–5 mg/kg).
Summary statistics: 10 adjustable parameters, AIC: 1.36.

Pharmacokinetic parameters		Pharmacodynamic parameters	
V_c (l/kg)	2.36 (2.57)	(A) Dose-independent parameters	
V_{ss} (l/kg)	8.82	E_0 (% baseline)	123.4 (7.10)
Cl (l h^{-1} kg^{-1})	5.66	*i*	3.49 (13.1)
$k_{(0,1)}$ (min^{-1})	0.036 (2.18)	k_{eo}(min^{-1})	0.018 (7.65)
$k_{(2,1)}$ (min^{-1})	0.049 (5.66)	(B) Dose-dependent parameters	
$k_{(1,2)}$ (min^{-1})	0.023 (5.97)	IC_{50}	
α (min^{-1})	0.101	Dose (mg/kg)	IC_{50} (μg/ml)
$t_{1/2}\,\alpha$ (min)	6.89	i.v. 1	0.041 (4.49)
β (min^{-1})	0.0084	i.v. 2.5	0.081 (6.45)
$t_{1/2}\,\beta$ (min)	81.8	i.v. 5	0.111 (9.25)
MRT (min)	93.5	Relative potencies	
Dose (mg/kg)	$AUC_{(0-\infty)}$ (μg min/ml)	Dose (mg/kg)	$IC_{50}^{1\ mg/kg}$/ $IC_{50}^{x\ mg/kg}$
i.v. 1 mg/kg	10.6	i.v. 1	1
i.v. 2.5 mg/kg	26.5	i.v. 2.5	0.506
i.v. 5 mg/kg	53.0	i.v. 5	0.369

a representative example, shows the observed and predicted profiles for the four animals (s1–s4) after 5 mg/kg clozapine administration. The active metabolite, *N*-desmethylclozapine, was not detected after administration of the 1 and 2.5 mg/kg doses. For the 5 mg/kg dose, the concentrations of *N*-desmethylclozapine in serum were low and ranged from 2 to 8 ng/ml (data not shown).

3.2.2. Group 2: intravenous 2.5 and oral 10 mg/kg clozapine

Serum concentration–time profiles and pharmacokinetic parameters for the intravenous 2.5 and oral 10 mg/kg clozapine doses are shown in Fig. 5C and Table 2 (left panel), respectively. Fig. 5D shows the observed and predicted profiles for the three animals (s5–s7) for the oral dose. The concentrations of *N*-desmethylclozapine in serum were low and ranged from 2 to 7 ng/ml after administration of the oral 10 mg/kg dose (data not shown). The absolute oral bioavailability (F) for oral clozapine was 5.32% (Table 2, left panel).

3.3. Pharmacokinetic–pharmacodynamic modeling

3.3.1. Group 1: i.v. 1–5 mg/kg clozapine

Fig. 6A shows the predicted (solid lines) and observed (filled circles) effect–time profiles of density of reinforcement for the three i.v. doses; a representative profile including data (ss1–ss4) for the 2.5 mg/kg dose is shown in Fig. 6B. The dose-independent parameters (E_0, k_{eo} and i) and associated errors are shown in Table 1A. One value of IC_{50} could not adequately describe the effects of clozapine across doses as judged by visual examination and the goodness of fit of the data assessed using AIC (AIC = 1.64). However, adequate descriptions of clozapine's effects for all three doses were attained when IC_{50} was allowed to vary as a function of dose (AIC = 1.36, Table 1). Table 1B shows the increase of IC_{50} with dose; as a result, the relative potency of the three doses, relative to the lowest dose (i.e., 1 mg/kg), decreases with increasing dose.

3.3.2. Group 2: i.v. 2.5 and p.o. 2.5–10 mg/kg clozapine

We combined the pharmacodynamic data from the three p.o. doses with the pharmacokinetic data of Group 2 in Model 2. Because one set of pharmacokinetic parameters could describe the concentration–time profiles for i.v. clozapine, we were able to simulate p.o. concentration–time profiles for the two lower doses (2.5–5 mg/kg; Group 2) using pharmacokinetic modeling. This facilitated the integration of pharmacokinetics with pharmacodynamics for all p.o. dose levels. The pharmacodynamic data from the i.v. 2.5 mg/kg dose was also reanalyzed with the simulated model by simultaneously optimizing the pharmacokinetic and pharmacodynamic data for the four doses. The dose-independent pharmacodynamic (E_0, k_{eo} and i) and dose-dependent (IC_{50}) parameters for the four doses are shown in Table 2A and B). The predicted effect–time profiles are indicated by solid lines in Fig. 6C; a representative profile including individual data (ss1–ss4) for the

Table 2
Clozapine pharmacokinetic and pharmacodynamic parameters (CV%) estimated by simultaneous pharmacokinetic–pharmacodynamic modeling of serum clozapine concentration–time profiles and density of reinforcement–time profiles after administration of clozapine (i.v. bolus 2.5 and p.o. bolus 2.5–10 mg/kg)
The superscript ''*x*'' denotes the respective clozapine doses (p.o. 2.5–10 and i.v. 2.5 mg/kg).
Summary statistics: 13 adjustable parameters, AIC: 3.23.

Pharmacokinetic parameters		Pharmacodynamic parameters	
V_c (l/kg)	2.11 (0.46)	(A) Dose-independent parameters	
V_{ss} (l/kg)	8.68	E_0 (% baseline)	133.5 (2.29)
Cl (l/h/kg)	4.65	*i*	3.87 (3.57)
$k_{(0,1)}$ (min^{-1})	0.033 (1.97)	k_{eo}(min^{-1})	0.108 (4.48)
$k_{(2,1)}$ (min^{-1})	0.076 (1.27)	(B) Dose-dependent parameters	
$k_{(1,2)}$ (min^{-1})	0.034 (1.65)	IC_{50}	
α (min^{-1})	0.134	Dose (mg/kg)	IC_{50} (μg/ml)
$t_{1/2}\,\alpha$ (min)	5.16	p.o. 2.5	0.011 (1.31)
β (min^{-1})	0.0082	p.o. 5	0.013 (2.45)
$t_{1/2}\,\beta$ (min)	84.2	p.o. 10	0.024 (2.14)
F (%)	5.32 (0.77)	i.v. 2.5	0.073 (1.54)
k_a (min^{-1})	0.030 (1.52)	Relative Potencies	
Dose (mg/kg)	$AUC_{(0-\infty)}$ (μg min/ml)	Dose (mg/kg)	$IC_{50}^{1\ mg/kg}$/ $IC_{50}^{x\ mg/kg}$
p.o. 2.5 mg/kg	1.62	p.o. 2.5	1
p.o. 5 mg/kg	3.24	p.o. 5	0.783
p.o. 10 mg/kg	6.48	p.o. 10	0.441
i.v. 2.5 mg/kg	32.3	i.v. 2.5	0.144

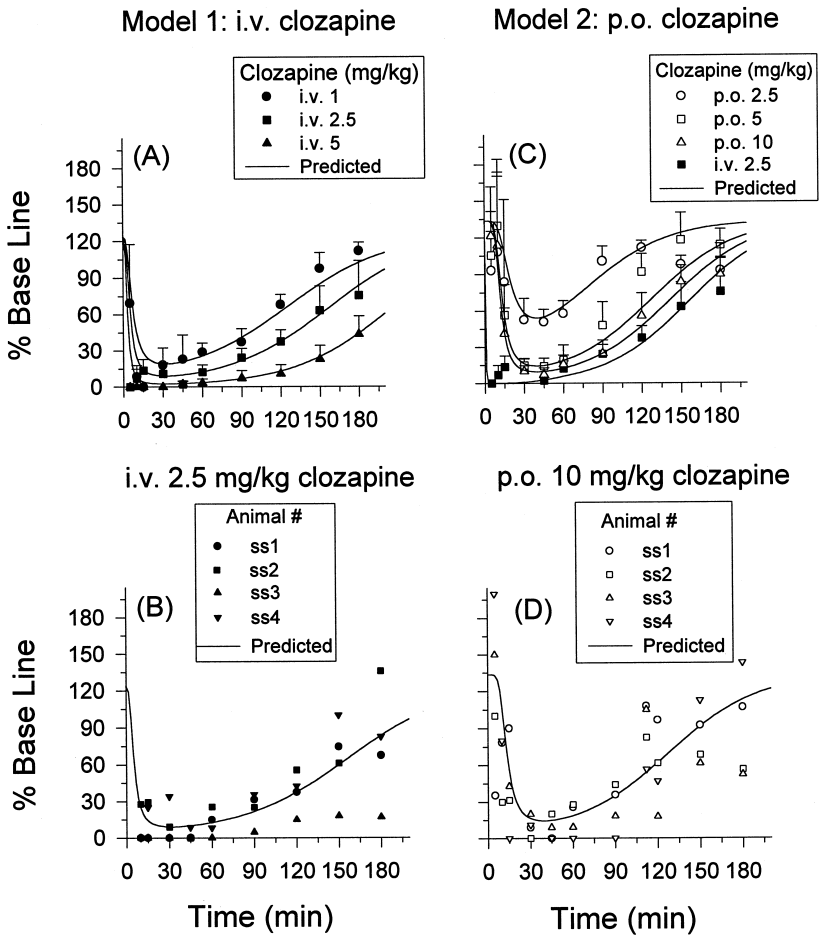

Fig. 6. Measured and predicted density of reinforcement–time profiles after i.v. administration of clozapine: (A) mean (S.E.M.) for 1–5 mg/kg; (B) individual animals (ss1–ss4) for the 2.5 mg/kg dose. Measured and predicted density of reinforcement–time profiles: (C) mean (S.E.M.) for the i.v. 2.5 and p.o. 2.5–10 mg/kg doses; (D) individual animals (ss1–ss4) for the p.o. 10 mg/kg dose.

p.o. 10 mg/kg dose is shown in Fig. 6D. Like Group 1, one IC_{50} value could not appropriately describe the i.v. and p.o. data as reflected by visual examination and the goodness of fit of the data (AIC = 4.05, which was greater than that shown in Table 2). Nevertheless, the IC_{50} value for the i.v. 2.5 mg/kg dose was somewhat similar to that estimated from Model 1. Relative to the p.o. 2.5 mg/kg dose, the relative potencies of p.o. clozapine decreased as a function of dose; however, the relative potency of the i.v. 2.5 mg/kg dose was the lowest (Table 2).

3.4. Effects of dose, $AUC_{(0-\infty)}$, and route of administration on the relative potencies of clozapine

Fig. 7A–C shows the relative potencies of i.v. and p.o. clozapine (Tables 1B and 2B) plotted against the logarithm of dose or $AUC_{(0-\infty)}$ for the density of reinforcement. The relative potency of i.v. clozapine decreased linearly and in parallel with those of p.o. clozapine with respect to the $AUC_{(0-\infty)}$, administered or bioavailable dose as indicated by the values of slope, R^2, and P (Fig. 7A–C). Although the dose range of oral clozapine (2.5–10 mg/kg) administered is greater than that for i.v. clozapine (1–5 mg/kg), $AUC_{(0-\infty)}$ values of the former were smaller than those of the latter because of the low F value of oral clozapine (5.32%). The administered p.o. doses of 2.5, 5 and 10 mg/kg were equivalent to bioavailable doses of 0.133, 0.266 and 0.532 mg/kg, respectively. This is why the curve of p.o. clozapine shifts to the left from that of i.v. clozapine (Fig. 7C) and in accordance with the curve plotted against $AUC_{(0-\infty)}$ (Fig. 7A).

To determine the effects of route of administration on the relative potency of clozapine, we calculated the potency values for the 1, 2.5 and 5 mg/kg i.v. clozapine doses relative to the p.o. 2.5 mg/kg dose; they were 0.268, 0.136 and 0.099, respectively. The relative potency of the i.v. 2.5 mg/kg dose closely followed the value of 0.144 estimated from Model 2 (Table 2). The relative potency of clozapine is solely dependent on the $AUC_{(0-\infty)}$ or dose that was bioavailable when the values of relative potency for all six doses relative to the p.o. 2.5 mg/kg dose were expressed (Fig. 7D–E). Collectively, the rela-

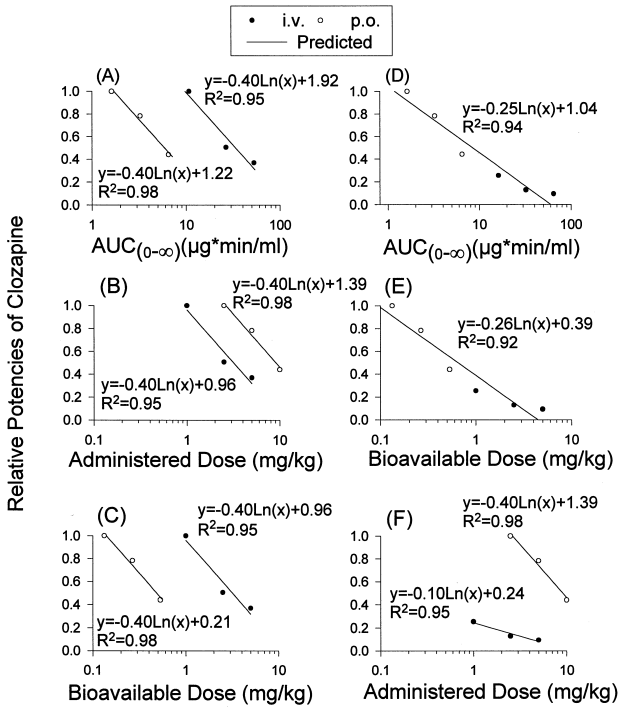

Fig. 7. Relations between potencies of clozapine relative to the lowest dose of each route of administration for the density of reinforcement and the logarithm of: (A) $AUC_{(0-\infty)}$; (B) administered dose; (C) bioavailable dose. Relations between potencies of clozapine relative to the p.o. 2.5 mg/kg dose and the logarithm of: (D) $AUC_{(0-\infty)}$; (E) bioavailable dose; (F) administered dose. The solid lines are the predicted values for the pharmacodynamic function. The equation and the associated statistics (R^2 values) for each regression analysis are included. All regressions attained the $P < 0.0001$ level.

tive potency of clozapine decreased significantly and linearly with the logarithm of $AUC_{(0-\infty)}$ or bioavailable dose, regardless of route of administration. Therefore, it becomes important that one adjusts the administered p.o. clozapine doses for F in order to examine the effects of route of administration on relative potency; otherwise, two independent curves are obtained as shown in Fig. 7F. The p.o. 2.5 mg/kg is the most efficacious dose used in this study.

3.5. Concentration–effect relations of density of reinforcement for i.v. and p.o. clozapine

Inasmuch as IC_{50} values were different across doses for each route of administration, three parallel curves of concentration–effect relation for density of reinforcements could be plotted for either i.v. or p.o. clozapine. The predicted effects are plotted against concentrations at the effect site for the i.v. 1 mg/kg dose (i.e., solid line) in Fig. 8A using the pharmacodynamic parameters shown in Table 1. Fig. 8A also shows that the mean observed effects across sessions for each i.v. dose (filled symbols) are plotted against the respective concentrations; however, these concentrations for the three i.v. doses have been corrected for the respective relative potencies. It is apparent that the concentration–effect relation of the i.v. 1 mg/kg dose can describe those of i.v. clozapine across doses if the relative potency of each dose was determined.

Likewise, Fig. 8B shows the predicted concentration–effect relation at the effect site for the p.o. 2.5 mg/kg dose (solid line) and the observed concentration–effect relations (open symbols) for p.o. clozapine (2.5–10 mg/kg). To include i.v. clozapine in the oral clozapine concentration–effect curve, the mean observed effects across sessions for each i.v. dose were plotted against the concentrations at

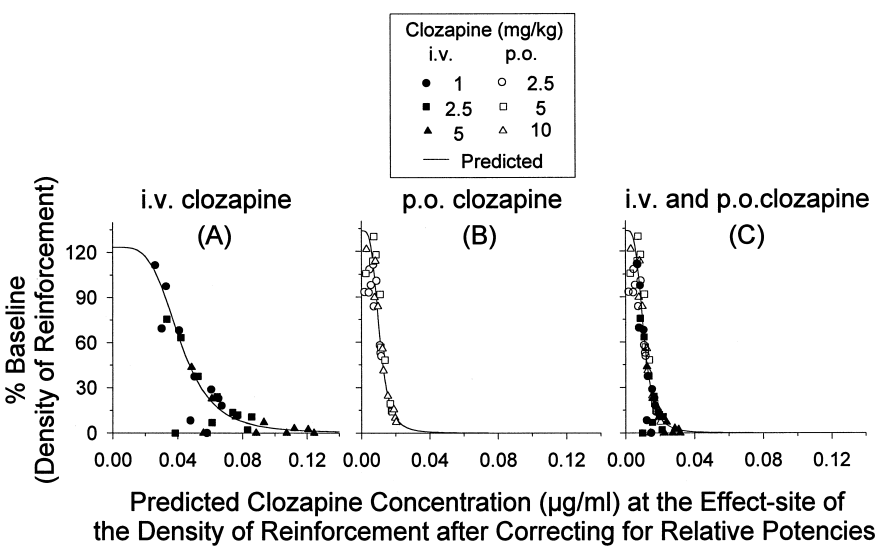

Fig. 8. Predicted and observed density of reinforcement plotted against clozapine concentrations at effect sites corrected for potencies of clozapine relative to the i.v. 1 mg/kg dose: (A) predicted (i.v. 1 mg/kg) and observed (i.v. 1–5 mg/kg). Predicted and observed density of reinforcement plotted against clozapine concentrations at effect sites corrected for potencies of clozapine relative to the p.o. 2.5 mg/kg dose: (B) predicted (p.o. 2.5 mg/kg) and observed (p.o. 2.5–10 mg/kg); (C) predicted (p.o. 2.5 mg/kg) and observed (i.v. 1–5 and p.o. 2.5–10 mg/kg).

the respective effect sites standardized against their relative potencies in relative to the p.o. 2.5 mg/kg dose but not to the i.v. 1 mg/kg dose (Fig. 8C). Collectively, the concentration–effect relation of the p.o. 2.5 mg/kg dose can describe those of clozapine across doses and routes if the relative potency for each dose was determined.

4. Discussion

This study presents the first attempt to simultaneously characterize the dose– and concentration–effect relations of i.v. and p.o. clozapine in rats. Pharmacokinetic parameters assessed for Group 2 were similar to those for Group 1 (Tables 1 and 2, left panels), indicating that between-group variability was minimal. Although $t_{1/2}\beta$ corresponded to previously found in rats (Baldessarini et al., 1993), values of both $t_{1/2}\beta$ and F for clozapine were much smaller than those reported in humans (Choc et al., 1990; Dain et al., 1997). Clozapine is extensively metabolized in humans (Stock et al., 1977; Dain et al., 1997). The two major metabolites are *N*-desmethylclozapine and clozapine-*N*-oxide; however, only the former metabolite is pharmacologically active (Kuoppamaki et al., 1993). In the present study, the low F value of oral clozapine (5.32%, Table 2) corresponded to its high extraction ratio (about 0.94) estimated from i.v. clozapine clearance (94.33 ml min^{-1} kg^{-1}, Table 1) and the hepatic blood flow of rats (assuming 100 ml min^{-1} kg^{-1}), suggesting that oral clozapine undergoes extensive hepatic first-pass metabolism. This means that upon oral administration, approximately 94% of the dose enters the body as metabolites. Because we only monitored serum clozapine and *N*-desmethylclozapine concentrations, the complete metabolic profiles were unknown in the present study.

Assessed by the values of $AUC_{(0-\infty)}$ or bioavailable dose, p.o. clozapine is more effective than i.v. clozapine in decreasing the density of reinforcement (Tables 1 and 2). In contrast, analyzed by dose–response determinations, i.v. clozapine was 6.9 times more potent than p.o. clozapine (Fig. 3A). This paradox derives from the two different approaches in determining drug potency for dose–response and concentration–effect relations. The former estimates, the ED_{50} from time-course profiles, collapsed into single points with no consideration of pharmacokinetics, whereas the latter estimates the IC_{50} from concentration–effect profiles. Furthermore, the absolute oral bioavailability is also helpful in adjusting administered doses to bioavailable doses, especially for drugs with low bioavailability. Estimation of ED_{50} from the effect–bioavailable-dose plot would have resulted in a different value than that of effect–administered-dose plot, but parallel to those of concentration–effect plots. Therefore, the pharmacodynamic parameters derived from the concentration–effect relation rather than dose–response relation appear to be more accurate. Due to the high correlation of IC_{50} and E_{max}, an added advantage in estimating drug potency by concentration–effect instead of dose–response determinations is the use of lower doses which avoid many of the adverse effects from higher doses. For example, for the density of reinforcement, the E_{max} was reached in concentration–effect curves (Fig. 8A–C), but not in dose–response curves (Fig. 3A).

Using IC_{50} as a marker of acute tolerance was first suggested by Hudson et al. (1983). We used this approach to demonstrate cocaine's differential effects on locomotor activity and operant behaviors (Lau et al., 1999b). In this context then, the increase in the IC_{50} with dose for both i.v. and p.o. clozapine may be explained by the phenomenon of acute tolerance. The IC_{50} may be considered an index for the sensitivity of behavioral performance to clozapine, and the relative potency and index for the extent of acute tolerance across doses and routes of administration. In this way, the extent of acute tolerance to clozapine varies directly with IC_{50} but inversely with relative potency; that is, as IC_{50} increases, acute tolerance also increases since relative potency has decreased. This bears direct application to estimating the extent of acute tolerance with dose and route of administration, as greater acute tolerance occurs after i.v. clozapine administration than p.o. administration. Although tolerance developed to clozapine's effects under chronic dose regimens (Waldmeier and Maitre, 1976; Sanger, 1985; Csernansky et al., 1993; Das and Fowler, 1995; Trevitt et al., 1998), acute tolerance to clozapine has not been previously shown in animals and humans. Hysteresis in the concentration–effect curve is generally used as an indicator for the occurrence of acute tolerance. In the present study, hysteresis was not apparent except at the 5-min time-point for the lowest i.v. dose as reflected in the effect–time profile (Fig. 6A). However, the decrease in relative potency with dose did provide a useful index for estimating the extent of acute tolerance to clozapine.

Metabolite profiles, as well as acute tolerance, can alter the values of IC_{50} for drugs after administration of different doses by various routes. In the present study, *N*-desmethylclozapine (an active metabolite) concentrations were low after the largest oral clozapine dose (10 mg/kg), which were comparable to those for the i.v. 5 mg/kg dose as described in Section 3. One would expect an even lower concentration for the two smaller oral clozapine doses (2.5 and 5 mg/kg). Thus, *N*-desmethylclozapine is unlikely to be involved in the increases in IC_{50} with dose for the two routes of administration because of their similarities in metabolite profiles. If indeed *N*-desmethylclozapine produced behavioral effects similar to clozapine, a different profile would likely be observed; that is, the IC_{50} would decrease with dose. Taken together, acute tolerance to clozapine's effects is the most plausible explanation for our results.

Numerous pharmacodynamic models have been used to demonstrate acute tolerance under various infusion-dose

regimens for psychoactive drugs in humans (e.g., Chow et al., 1985; Kroboth et al., 1993) and animals (e.g., Ekblom et al., 1993). We have tried to use some of these models; however, they did not describe our data well. To our knowledge, the extent of acute tolerance has not been characterized following bolus administration via i.v. and extravascular routes with pharmacodynamic models. In fact, a tolerance model developed for morphine under constant infusion could not totally account for both the rate and extent of acute tolerance in the same study after an i.v. bolus dose (Ekblom et al., 1993). This may be due to differences in the rate of tolerance development for the two dose regimens, such as a possible fast and slow tolerance component (Brase et al., 1976). Therefore, models describing slower tolerance development under constant infusion dose regimens may not be able to account for the possible rapid tolerance development under a bolus dose regimen (Ekblom et al., 1993).

Rate of drug administration has been suggested as an important determinant of both the clinical effects and development of acute tolerance to benzodiazepines (Greenblatt et al., 1977; Ellinwood et al., 1985). Indeed, the effects of rates of drug administration on pharmacodynamics have been demonstrated in humans (Wakelkamp et al., 1998) and in rats (Cleton et al., 1999). In the present study, the rates of input or absorption of clozapine differed markedly with both routes of administration and dose size. The rate of absorption for the p.o. 10 mg/kg dose was considerably slower (about 0.00115 mg/s) on the basis of its k_a value (Table 2) than the rates of input for the three i.v. doses (0.011–0.053 mg/s), although the absorption rate of p.o. clozapine could not be precisely determined from the present study. Assuming constant k_a, absorption rates for the two lower p.o. doses would likewise be even slower. Because IC_{50} varied directly with rates of input across doses and routes of administration of clozapine, less acute tolerance was seen with the slowest input rate than with more rapid input rates, findings consistent with a previous frusemide study (Wakelkamp et al., 1998). The input rate of clozapine seems to explain the effects of dose and route of administration on the extent of acute tolerance to clozapine. The rate of input may not be as crucial in acute tolerance development to other drugs, however. For example, acute tolerance to midazolam was not developed with three different rates of administration in rats (Mandema et al., 1991). Using the same acute-dose regimen under the differential reinforcement of low rate 45-s schedule, acute tolerance may explain the potency differences between i.v. and p.o. cocaine (Ma et al., 1999), but it was not evident in i.v. cocaine with different input rates (Lau et al., 1999a). It is unlikely that a preceding dose had residual effects on succeeding doses, which were separated by 3–5 days in our behavioral paradigm; otherwise, acute tolerance to clozapine would have been greater for p.o. clozapine than i.v. clozapine, as the i.v. dose series was administered prior to the p.o. dose series.

Although clozapine is used as an antipsychotic agent, it possesses pharmacological (De Maio, 1972; Ellinwood et al., 1983) and behavioral (e.g., Canon, 1979; Spealman et al., 1983) characteristics similar to benzodiazepines. According to 3-h collapsed data, clozapine, resembling the effects of benzodiazepines (e.g., alprazolam, midazolam) and cocaine, increased the shorter-response rate and decreased the density of reinforcement (Fig. 3A–B; Lau et al., 1998, 1999a). However, two distinct patterns were seen in effect–time profiles of shorter-response rates, although the profile for density of reinforcement was similar across these drugs (Lau and Heatherington, 1997; Lau et al., 1998, 1999a). Thus, the shorter-response rate profile may be said to reflect the drug's putative pharmacological action (e.g., stimulatory, sedative), while density of reinforcement profile is an index of timing performance under the differential reinforcement of low rate 45-s schedule. The increased shorter-response rate is the stimulatory effect reported herein. Following i.v. administration, for example, cocaine increased the shorter-response rate immediately, whereas alprazolam increased the shorter-response rate at a later time. This observation is consistent with their putative pharmacological actions; cocaine is a psychomotor stimulant and alprazolam is a sedative agent, despite both drugs showing a similar pattern of disappearance in serum drug concentration via i.v. administration. However, two peaks were observed in the shorter-response rate–time profiles for the two benzodiazepines after s.c. administration due to the difference in onset of the stimulatory and sedative effects (Lau and Heatherington, 1997; Lau et al., 1998). We used the stimulation–sedation model to characterize the effect–time profiles of the two benzodiazepines. The first peak occurred before onset of the sedative effect, while the second occurred after offset of the sedative effect; however, the first peak was absent for i.v. alprazolam due to the rapid effect onset by the i.v. route. Following i.v. and p.o. clozapine, the first peak is absent at the corresponding alprazolam site, while the observed peak for each dose is comparable to the second alprazolam peak in location (Fig. 4A–B). This could result from differences in the rate and extent of the stimulatory and sedative effects produced by clozapine and the two benzodiazepines; as a result, the first peak is absent for clozapine. The same stimulation–sedation model could be used to characterize the effect–time profiles of i.v. and p.o. clozapine (unpublished data), implying that effects of clozapine on shorter-response rates were similar to those of the two benzodiazepines.

In summary, the behavioral and pharmacological profiles of clozapine can be discriminated from those characterizing benzodiazepines through behavioral and pharmacokinetic–pharmacodynamic analyses. Acute tolerance was evident in concentration–effect relations of clozapine, but not in dose–response or effect–time profiles. The extent of acute tolerance was dependent on bioavailable dose or concentration regardless of route of administration. A sin-

gle concentration–effect relation described effects of clozapine on the density of reinforcement for timing behavior as a pharmacodynamic endpoint across the doses and routes used. Increases in IC_{50} with dose of clozapine correspond to decreases in efficacy in concentrations at effect sites, indicating that acute tolerance development is a continuous, dynamic process and provides a suitable basis for investigation of the in-vivo pharmacodynamics of clozapine. Furthermore, effects of dose and routes of administration on acute tolerance presented herein may have direct implications in optimizing therapeutic dose regimens for clozapine.

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References

Baldessarini, R.J., Centorrino, F., Flood, J.G., Volpicelli, S.A., Huston-Lyons, D., Cohen, B.M., 1993. Tissue concentrations of clozapine and its metabolites in the rat. Neuropsychopharmacology 9, 117–124.

Brase, D.A., Iwamoto, E.T., Loh, H.H., Way, E.L., 1976. Reinitiation of sensitivity to naloxone by single narcotic injection in postaddicted mice. J. Pharmacol. Exp. Ther. 197, 317–325.

Canon, J.G., 1979. A comparison of clozapine, chlorpromazine, and thioridazine upon DRL performance in the Squirrel Monkey. Psychopharmacology 64, 55–60.

Canon, J.G., Lippa, A.S., 1977. Effects of clozapine, chlorpromazine and diazepam upon adjunctive and schedule controlled behaviors. Pharmacol. Biochem. Behav. 6, 581–587.

Choc, M.G., Hsuan, F., Honigfeld, G., Robinson, W.T., Ereshevsky, L., Crismon, M.L., Saklad, S.R., Hirschowitz, J., Wagner, R., 1990. Single- vs. multiple-dose pharmacokinetics of clozapine in psychiatric patients. Pharm. Res. 7, 347–351.

Chow, M.J., Ambre, J.J., Ruo, T.I., Atkinson, A.J., Bowsher, D.J., Fishman, M.W., 1985. Kinetics of cocaine distribution, elimination and chronotropic effects. Clin. Pharmacol. Ther. 38, 318–324.

Cleton, A., Mazee, D., Voskuyl, R.A., Danhof, M., 1999. Rate of change of blood concentrations is a major determinant of the pharmacodynamics of midazolam in rats. Br. J. Pharmacol. 127, 227–235.

Csernansky, J.G., Wrona, C.T., Bardgett, M.E., Early, T.S., Newcomer, J.W., 1993. Subcortical dopamine and serotonin turnover during acute and subchronic administration of typical and atypical neuroleptics. Psychopharmacology 110, 145–151.

Dain, J.G., Nicoletti, J., Ballard, F., 1997. Biotransformation of clozapine in humans. Drug Metab. Dispos. 25, 603–609.

Das, S., Fowler, S.C., 1995. Acute and subchronic effects of clozapine on licking in rats: tolerance to disruptive effects on number of licks, but no tolerance to rhythm slowing. Pyschopharmacology 120, 249–255.

DeLean, A., Munson, P.J., Guardabasso, V., Rodbard, D., 1992. A User's Guide to ALLFIT. Simultaneous fitting of families of sigmoidal dose response curves using the four-parameter logistic equation. Lab. Theoret. Physical Biology, Natl. Inst. Child Health and Human Development, NIH, Bethesda, MD, USA.

De Maio, D., 1972. Clozapine, a novel major tranquilizer. Arzneim.-Forsch. 22, 919–921.

Ekblom, M., Hammarlund-Udenaes, H., Paalzow, L., 1993. Modeling of tolerance development and rebound effect during different intravenous administration of morphine to rats. J. Pharmacol. Exp. Ther. 266, 244–252.

Ellinwood, E.H. Jr., Linnoila, M., Easler, M.E., Molter, D.W., 1983. Profile of acute tolerance to three sedative anxiolytics. Psychopharmacology 79, 137–141.

Ellinwood, E.H. Jr., Heatherly, D.G., Nkaido, A.M., Bjornsson, T.D., Kilts, C., 1985. Comparative pharmacokinetics and pharmacodynamics of lorazepam, alprazolam and diazepam. Psychopharmacology 86, 392–399.

Fitton, A., Heel, R.C., 1990. Clozapine: a review of its pharmacological properties and therapeutic use in schizophrenia. Drugs 40, 722–747.

Fowler, S.C., Liou, J., 1998. Haloperidol, raclopride and eticlorpride induce microcatalepsy during operant performance in rats, but clozapine and SCH 23390 do not. Psychopharmacology 140, 81–90.

Greenblatt, D.J., Shader, R.I., Harmatz, J.S., Franke, K., Koch-Weser, J., 1977. Absorption rate, blood concentrations and early response to oral chlordiazepoxide. Am. J. Psychiatry 134, 559–562.

Hudson, R.J., Stanski, D.R., Saidman, L.J., Meathe, E., 1983. A model for studying depth of anesthesia and acute tolerance to thiopental. Anesthesiology 59, 302–308.

Jann, M.W., Grimsley, S.R., Gray, E.C., Chang, W.H., 1993. Pharmacokinetics and pharmacodynamics of clozapine. Clin. Pharmacokinet. 24, 161–176.

Kroboth, P.D., Bertz, R.J., Smith, R.B., 1993. Acute tolerance to triazolam during continuous and step infusions: estimation of the effect offset rate constant. J. Pharmacol. Exp. Ther. 264, 1047–1055.

Kuoppamaki, M., Syvalahti, E., Hietala, J., 1993. Clozapine and *N*-desmethlclozapine are potent 5-HT_{IC} receptor antagonists. Eur. J. Pharmacol. 245, 179–182.

Lau, C.E., Heatherington, A.C., 1997. Pharmacokinetic–pharmacodynamic modeling of stimulatory and sedative effects of alprazolam: timing performance deficits. J. Pharmacol. Exp. Ther. 283, 1119–1129.

Lau, C.E., Wang, J., 1996. Alprazolam, caffeine and their interaction: relating DRL performance to pharmacokinetics. Psychopharmacology 126, 115–124.

Lau, C.E., Ma, F., Wang, Y., Smith, C., 1996. Pharmacokinetics and bioavilability of midazolam after intravenous, subcutaneous, intraperitoneal and oral administration under a chronic food-limited regimen: relating DRL performance to pharmacokinetics. Psychopharmacology 126, 241–248.

Lau, C.E., Wang, Y., Falk, J.L., 1997. Differential reinforcement of low rate performance, pharmacokinetics and pharmacokinetic–pharmacodynamic modeling: independent interaction of alprazolam and caffeine. J. Pharmacol. Exp. Ther. 281, 1013–1029.

Lau, C.E., Wang, Y., Ma, F., 1998. Pharmacokinetic–pharmacodynamic modeling of the coexistence of stimulatory and sedative components for midazolam. Eur. J. Pharmacol. 346, 131–144.

Lau, C.E., Ma, F., Foster, D.M., Falk, J., 1999a. Pharmacokinetic–pharmacodynamic modeling of the psychomotor stimulatn effect of cocaine after intravenous administration: timing performance deficits. J. Pharmacol. Exp. Ther. 288, 535–543.

Lau, C.E., Wang, Y., Sun, L., Lobarinas, E., Wang, Q., Nguyen, K., Falk, J.L., 1999b. Pharmacokinetic determinants of cocaine's differential effects on locomotor and operant behavior. Eur. J. Pharmacol. 381, 85–92.

Ma, F., Lau, C.E., 1998. Determination of clozapine and its metabolite, *N*-desmethylclozapine, in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats. J. Chromatogr., B 712, 193–198.

Ma, F., Falk, J.L., Lau, C.E., 1999. Cocaine pharmacodynamics after

intravenous and oral administration in rats: relation to pharmacokinetics. Psychopharmacology 144, 323–332.

Mandema, J.W., Tukker, E., Danhof, M., 1991. Pharmacokinetic–pharmacodynamic modeling of the EEG effects of midazolam in individual rats: influence of rate and route of administration. Br. J. Pharmacol. 102, 663–668.

Meltzer, H.Y., Matusbara, S., Lee, J.C., 1989. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D-1, D-2 and serotonin$_2$ pK_i values. J. Pharmacol. Exp. Ther. 251, 238–246.

Richelson, E., 1984. Neuroleptic affinities for human brain receptors and their use in predicting adverse effects. J. Clin. Psychiatry 45, 331–336.

Sanger, D.J., 1985. The effects of clozapine on shuttle-box avoidance responding in rats: comparisons with haloperidol and chlordiazepoxide. Pharmacol. Biochem. Behav. 23, 231–236.

Schaefer, G.J., Michael, R.P., 1980. Acute effects of neuleptics on brain self-stimulation thresholds in rats. Psychopharmacology 67, 9–15.

Seiden, L.S., Dahms, J.L., Shaughnessy, R.A., 1985. Behavioral screen for antidepressants: the effects of drugs and electroconvulsive shock on performance under a differential-reinforcement-of-low-rate schedule. Psychopharmacology 86, 55–60.

Sheiner, L.B., Stanski, D.R., Vozeh, S., Miller, R.D., Ham, J., 1979. Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to D-tubocurarine. Clin. Pharmacol. Ther. 25, 358–370.

Spealman, R.D., Katz, J.L., 1980. Some effects of clozapine on punished responding by mice and Squirrel monkeys. J. Pharmacol. Exp. Ther. 212, 435–440.

Spealman, R.D., Kelleher, R.T., Goldberg, S.R., DeWeese, J., Goldberg, D.M., 1983. Behavioral effects of clozapine: comparison with thioridazine, chlorpromazine, haloperidol and chlordiazepoxide in Squirrel monkeys. J. Pharmacol. Exp. Ther. 224, 127–134.

Stille, G., Lauener, H., Eichenberger, E., 1971. The pharmacology of 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo(b,e) (1,4) diaepine (clozapine). Farmaco, Ed. Sci. 26, 603–625.

Stock, V.B., Spiteller, G., Heipertz, R., 1977. Austausch aroomatisch Gehinderter Halogens Gegen OH- and SCH_3-bei der metabolisierung des clozapine in Menschlichen Korper. Arzneim.-Forsch. 27, 982–990.

Trevitt, J., Atherton, A., Aberman, J., Salamone, J.D., 1998. Effects of subchronic administration of clozapine, thioridazine and haloperidol on tests related to extrapyramidal motor function in the rat. Psychopharmacology 137, 61–66.

Wakelkamp, M., Alvan, G., Scheinin, H., Gabrielsson, J., 1998. The influence of drug input rate on the development of tolerance to frusemide. Br. J. Clin. Pharmacol. 46, 479–487.

Waldmeier, P.C., Maitre, L., 1976. Clozapine: reduction of the initial dopamine turnover increase by repeated treatment. Eur. J. Pharmacol. 38, 197–203.

Wang, Y., Lau, C.E., 1998. Caffeine has similar pharmacokinetics and behavioral effects via the IP and PO routes of administration. Pharmacol. Biochem. Behav. 60, 271–278.

Wenger, G.R., 1979. Effects of clozapine, chlorpromazine and haloperidol on schedule-controlled behavior. Pharmacol. Biochem. Behav. 11, 661–667.