Determination of minimal steady-state plasma level of diazepam causing seizure threshold elevation in rats

Ashish Dhir1 | Michael A. Rogawski1,2

1 Department of Neurology, School of Medicine, University of California, Davis, Sacramento, CA, USA
2 Department of Pharmacology, School of Medicine, University of California, Davis, Sacramento, CA, USA

Correspondence
Michael A. Rogawski, Department of Neurology, School of Medicine, University of California, Davis, Sacramento, CA, USA.
Email: rogawski@ucdavis.edu

Funding information
CounterACT Program; National Institutes of Health Office of the Director; National Institute of Neurological Disorders and Stroke, Grant/Award Number: NS079202; Acorda Therapeutics

Summary

Objective: Diazepam, administered by the intravenous, oral, or rectal routes, is widely used for the management of acute seizures. Dosage forms for delivery of diazepam by other routes of administration, including intranasal, intramuscular, and transbuccal, are under investigation. In predicting what dosages are necessary to terminate seizures, the minimal exposure required to confer seizure protection must be known. Here we administered diazepam by continuous intravenous infusion to obtain near–steady-state levels, which allowed an assessment of the minimal levels that elevate seizure threshold.

Methods: The thresholds for various behavioral seizure signs (myoclonic jerk, clonus, and tonus) were determined with the timed intravenous pentylenetetrazol seizure threshold test in rats. Diazepam was administered to freely moving animals by continuous intravenous infusion via an indwelling jugular vein cannula. Blood samples for assay of plasma levels of diazepam and metabolites were recovered via an indwelling cannula in the contralateral jugular vein.

Results: The pharmacokinetic parameters of diazepam following a single 80-μg/kg intravenous bolus injection were determined using a noncompartmental pharmacokinetic approach. The derived parameters Vd, CL, t1/2a (distribution half-life) and t1/2b (terminal half-life) for diazepam were, respectively, 608 mL, 22.1 mL/min, 13.7 minutes, and 76.8 minutes, respectively. Various doses of diazepam were continuously infused without or with an initial loading dose. At the end of the infusions, the thresholds for various behavioral seizure signs were determined. The minimal plasma diazepam concentration associated with threshold elevations was estimated at approximately 70 ng/mL. The active metabolites nordiazepam, oxazepam, and temazepam achieved levels that are expected to make only contributions to the threshold elevations.

Significance: Diazepam elevates seizure threshold at steady-state plasma concentrations lower than previously recognized. The minimally effective plasma concentration provides a reference that may be considered when estimating the diazepam exposure required for acute seizure treatment.

KEYWORDS
continuous infusion, diazepam, pharmacokinetics, seizure threshold, time intravenous pentylenetetrazol seizure test
1 | INTRODUCTION

Diazepam is a long-acting benzodiazepine that is often prescribed for its anxiolytic, sedative-hypnotic, and muscle relaxant properties.1 In addition, diazepam is an effective antiseizure agent that is used in the acute treatment of seizure exacerbations, seizure clusters (acute repetitive seizures), and status epilepticus.2–4 As a class, benzodiazepines act as positive allosteric modulators of GABA A receptors, which are GABA-gated chloride channels that are the principal mediators of fast synaptic inhibition in the central nervous system.2,5–7 Diazepam is administered by the oral, intravenous, intramuscular, and rectal routes.3 In the United States, the only product approved for the out-of-hospital acute treatment of seizures is the diazepam rectal gel formulation dispensed in a proprietary rectal delivery system. The rectal gel is indicated for intermittent use to control bouts of increased seizure activity. Alternative, more convenient or more rapidly acting formulations intended for other routes of administration could replace the rectal formulation in managing acute and repetitive seizure attacks, provided they deliver adequate levels of diazepam required for antiseizure activity.8 Intranasal and buccal formulations have been investigated.9,10

Although diazepam has been used clinically for more than 50 years and its pharmacokinetic properties have been studied extensively in preclinical and clinical studies,11 little information exists on the minimal blood concentration required for its antiseizure effect. In experimental studies in animal models, diazepam is typically administered by bolus and the levels fall rapidly during the time of antiseizure testing. The rate of fall in blood and brain concentrations is particularly rapid following a bolus in a naive animal due to redistribution. Therefore, it has not been possible to obtain reliable estimates of the blood concentrations required for antiseizure activity. In one such study in unanesthetized rats, Marcucci et al12 observed that concentrations above 150 ng/mL were associated with antiseizure activity. However, in unanesthetized cats with a penicillin focus, Celesia et al13 estimated that diazepam concentrations above 170 ng/mL were required to inhibit pentylenetetrazol (PTZ)-evoked and spontaneous electrographic seizures.

Human studies have also resulted in a range of estimates. Based on levels achieved after bolus intravenous administration, it has been proposed that serum levels above 400 ng/mL are required for anticonvulsant effects.11,14 Some investigators have used electrographic measures to assess antiseizure activity. Photoconvulsive responses and spontaneous epileptiform discharges were suppressed in one study with concentrations, respectively, in the range of 100-500 ng/mL and 500-1400 ng/mL.15 In a separate study, interictal spike activity was reduced with serum levels between 80 and 410 ng/mL (mean 210 ng/mL),16 whereas in another study in children, serum concentrations greater than 250 ng/mL were associated with a decrease in paroxysmal activity on electroencephalography (EEG).17 A direct correspondence between effects on electrographic measures and prevention of clinical seizures has not been established. Estimates of the threshold plasma concentrations of diazepam required to terminate or prevent behavioral seizures are available in only a limited number of instances. In a 15-year-old girl with focal epilepsy who received a bolus intravenous diazepam infusion, plasma levels >130 ng/mL were associated with seizure freedom.18 In another report, a child in whom seizures had been arrested by diazepam had recurrence of convulsions when diazepam plasma levels dropped to 130 ng/mL.19 However, in one adult with severe refractory seizures occurring in clusters, seizures recurred after rectal administration of diazepam when serum concentrations would have been above 170 ng/mL.16

Given the uncertainties in attempts to estimate seizure threshold values following bolus dosing or when patients are experiencing the unpredictable occurrence of spontaneous seizures, we sought to establish in rats the minimal steady-state concentration of diazepam in plasma that elevates the seizure threshold by using an approach where redistribution is complete and blood levels are maintained at a near-constant concentration. Both the left and right jugular veins of the rats were permanently catheterized to allow continuous infusion of diazepam into one jugular and withdrawal of well-mixed blood for plasma level determinations from the other jugular. In an initial series of experiments, we characterized the pharmacokinetic parameters of diazepam and its metabolites following a bolus dose using

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**Key Points**

- Various dosage forms of diazepam are under development to treat acute seizures, but target blood levels are poorly defined.
- Estimates of levels conferring seizure protection in animals and humans have been based on studies in which levels are rapidly changing.
- To obtain a more reliable estimate, continuous infusion in rats was used to maintain the concentration at near-steady-state levels.
- Seizure threshold was assessed using the timed intravenous pentylenetetrazol seizure test.
- Plasma concentrations of diazepam greater than about 70 ng/mL were found to elevate the seizure threshold.
a noncompartmental analysis approach. The derived parameter estimates allowed us to plan the dosing regimens to be applied in the remainder of the study. We used the following 2 dosing paradigms to achieve near-steady-state diazepam plasma concentrations: (1) continuous intravenous infusion of diazepam for 4 half-lives, which is expected to bring the diazepam plasma concentration to 94% of the steady-state level, and (2) a loading dose bolus followed by continuous intravenous infusion for 1 half-life, which allowed a near-steady-state level of diazepam to be achieved more rapidly. In both dosing paradigms, exposure to diazepam was sufficiently prolonged so that redistribution was complete and blood levels were not changing rapidly during the time of seizure threshold measurement. The timed intravenous PTZ seizure threshold test was used to quantify the extent of elevation of the seizure threshold. This test is a sensitive measure of the antiseizure activity of drugs that act as positive modulators of GABA<sub>A</sub> receptors, such as benzodiazepines, including diazepam. In the protocol applied in our laboratory, the seizure threshold values are determined for 3 different behavioral seizure signs—myoclonic jerks, clonus, and tonus—that usually occur sequentially as the total dose of diazepam infused increases.

2 | MATERIALS AND METHODS

2.1 | Animals

Male Sprague-Dawley rats (225-400 g) obtained from Charles River Laboratories were kept in a vivarium under controlled environmental conditions with an artificial 12-h light/dark cycle. Animals were allowed to acclimatize in the vivarium at least 3 days before the beginning of any experimental procedure. Experiments were performed during the light phase of the light/dark cycle after a 30-minute period of acclimation to the experimental laboratory. The animal facilities were fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care. All studies were performed under protocols approved by the Institutional Animal Care and Use Committee of the University of California, Davis, in strict compliance with the Guide for the Care and Use of Laboratory Animals of the National Research Council (National Academy Press, Washington, DC). The principles outlined in the Basel declaration (http://www.basel-declaration.org) including the 3R concept were considered when planning the experiments.

2.2 | Diazepam and vehicle solutions

A commercially available formulation of diazepam (5 mg/mL diazepam injection, USP solution; Hospira Inc., Lake Forest, IL, USA) was used in the present study. Diazepam is not water-soluble and is formulated in an aqueous vehicle containing 40% propylene glycol, 10% alcohol, 5% sodium benzoate and benzoic acid, and 1.5% benzyl alcohol. To dilute the commercial diazepam formulation, we used a solution of the same composition as the vehicle in the commercial preparation. This vehicle was administered in some experiments as control. The pH of the diazepam or vehicle solution was maintained between 6.2 and 6.5 as in the commercial formulation.

2.3 | Catherization of the jugular vein

Animals were anesthetized using the combination of ketamine (50-100 mg/kg, ip) and dexmedetomidine (0.5 mg/kg, ip). The ventral portion of the neck was shaved and an incision was made starting at the base of the neck and extending to the chest at the clavicle. The external right and left jugular veins were isolated and catheterized using polyurethane tubing (0.025 in × 0.0140 in; Instech Laboratories Inc., Plymouth Meeting, PA, USA). The patency of the cannula was confirmed by injecting 200-250 µL of sterile saline followed by a slow withdrawal of the blood. After the patency of the cannula was confirmed, the cannula was tied securely to the vein with caudal and rostral sutures; both were positioned over the polyurethane overlap to avoid occlusion of the tubing. The cannulae were exteriorized at the back of the neck. The catheters were locked with heparin (500 U/mL)-dextrose catheter lock solution (SAI Infusion Technologies, Lake Villa, Illinois, USA). The exteriorized end of the catheters was sealed with a stainless-steel plug. The anesthesia was reversed using atipamezole hydrochloride (1 mg/kg, ip; Antisedan, Orion Corporation, Espoo, Finland). The animals were allowed to recover from the surgery for at least 7 days before testing. During these 7 days, the cannulae were flushed occasionally with the heparin solution using a blunt needle (23 G) and 1 mL syringe to prevent blocking by clots. The instilled solution was withdrawn, aspirated fluid/blood was discarded, and the cannula was flushed with saline to clear it of blood and refilled with fresh heparin-dextrose lock solution.

2.4 | Timed intravenous PTZ seizure threshold test

To evaluate the effect of diazepam on seizure threshold, the GABA<sub>A</sub>-receptor antagonist PTZ was infused via a jugular vein catheter and the doses required to elicit various seizure signs were determined. PTZ (20 mg/mL) was infused at a constant rate of 0.25 mL/min using a Becton Dickinson 5 mL syringe mounted on an infusion pump (Model “11” plus syringe pump; Harvard Apparatus,
Holliston, MA). The thresholds to the following endpoints were determined: (1) the first myoclonic jerk, (2) the onset of generalized clonus with loss-of-righting reflex, and (2) the onset of tonic phase. The infusion was stopped at the onset of tonic phase. Latencies were measured from the start of the PTZ infusion to the onset of each behavior. The PTZ threshold (minimum) dose (in mg/kg) to achieve each endpoint was determined according to the following formula: (infusion duration [s] × infusion rate [mL/min] × convulsant drug concentration [mg/mL] × 1000)/ (60 [s] × weight of rat [g]).

2.5 | Blood collection and assay

Blood was collected by retroorbital sinus puncture in K2-EDTA tubes. The whole blood was centrifuged at 2000 RPM for 10 minutes at 4°C, and the clear plasma supernatant was collected and stored at −80°C until analyzed. Plasma diazepam and metabolite levels were determined using a validated liquid chromatographic and tandem mass spectrometric assay (LC-MS/MS; Covance Laboratories, Durham, NC). In the case of diazepam and nordiazepam, the assay had a lower limit of quantitation of 1 ng/mL and was validated for concentrations up to 500 ng/mL with standards. In the case of oxazepam and temazepam, the assay was validated for concentrations in the range of 0.1-50 ng/mL.

2.6 | Pharmacokinetic and statistical analysis

Pharmacokinetic analysis was conducted with Kinetica software (ThermoFisher Scientific).

Results are expressed as mean ± standard error of the mean (SEM); the significance of the difference in the responses of treatment groups with respect to control is based on 1-way analysis of variance (ANOVA) followed by specific post hoc comparisons using Tukey’s test. Differences were considered statistically significant when the probability of type I error was <0.05.

3 | RESULTS

3.1 | Determination of pharmacokinetic parameters of diazepam and metabolites after intravenous bolus administration of diazepam in rats

Diazepam (80 μg/kg) was administered by intravenous bolus injection over 5 seconds via the lateral tail vein in 8 rats. For each rat, blood was collected at intervals from 5 to 240 minutes following the bolus and the concentrations of diazepam, nordiazepam, temazepam, and oxazepam were determined in the plasma. As shown in Figure 1, the mean diazepam plasma concentration fell monotonically over time during the 240-minute period following injection. At the last time-point sampled (240 minutes), diazepam could be detected in only 3 of the 8 animals; the levels in the remainder were below the limit of detection. The metabolite levels overall were much lower than the levels of diazepam; levels of the des-methyl congeners nordiazepam (nordiazepam), considered to be the principal metabolite of diazepam, were modestly greater than those of the 3-hydroxy metabolites oxazepam and temazepam. These latter 2 metabolites showed a delayed rise. Nordiazepam was detected in 3, 8, 6, and 2 of 8 animals at 5, 10, 30, and 60 minutes, respectively, and in none of the animals at the later time points. The levels of temazepam in rat plasma, although low, could be detected in 8 of 8 animals at the 5-60 minutes time points, and in 4, 2, and 0 of 8 animals at 90, 120, and 240 minutes, respectively. Like diazepam, oxazepam was detectable in all animals at all time points except at the 240-minute time point when levels were detectable in only 3 of 8 animals.

Noncompartmental analysis (Kinetica software; ThermoFisher) was used to calculate the following pharmacokinetic parameters from plasma diazepam levels in each of the 8 rats: maximum observed plasma concentration (Cmax), area under the curve for time zero to last sampling time (AUC0–240), initial (disposition) and elimination half-lives (t1/2α and t1/2β), volume of distribution (Vd), clearance (CL), and elimination rate constant k₂. These values are provided in Table 1.

3.2 | Effect of continuous intravenous infusion of diazepam on diazepam and metabolite levels and seizure sign thresholds

Assuming first-order kinetics, the infusion rate R to achieve a steady-state concentration Cs is given by R = Cs × CL. To achieve near Cs of 89, 200, 400 ng/mg with CL = 22.14 mL/min implies R ~ 2, 4.5, and 9 μg/min. (The maximum target concentration is approximately the peak diazepam concentration achieved by diazepam rectal gel as specified in the US Food and Drug Administration (FDA) approved label.) Continuous intravenous infusion of diazepam at these rates via the right jugular vein (flow rate in all cases was 1 mL/h) for a total of 308 minutes resulted in mean ± SEM plasma levels of 55.0 ± 9.1, 130.1 ± 15.5, and 360 ± 55 ng/mL, respectively (Figure 2A). Regression of mean plasma level achieved vs infusion rate revealed a linear relationship with slope of 0.044 min/mL and intercept −48 μg/min (r² = 0.99). The levels of the 3 diazepam metabolites also generally rose in a corresponding fashion, but the absolute concentrations were 10-fold less than those of the parent (Figure 2B-D), consistent with a prior study that found diazepam to...
undergo only limited metabolism in the rat (Marcucci et al.12).

The intravenous PTZ infusion test was conducted at the end of the 308-minute infusion for vehicle and each of the 3 infusion rates. We have found that the vehicle elevated the threshold for all 3 seizure signs, with greater effect on clonus and tonus than on myoclonic jerks. Vehicle was demonstrated to elevate the threshold for tonus (see Figure 3), which is likely due to the various alcohols that are included to solubilize diazepam. To account for this effect, we assessed changes in threshold with respect to vehicle treatment. Statistically significant elevations in all 3 seizure endpoints were observed with doses of 4.5 and 9 µg/min but not 2 µg/min. Thus, seizure threshold elevation was obtained with an uncorrected mean concentration of 130 ng/mL (achieved by continuous infusion of 4.5 µg/min) but not 55 ng/mL (achieved by continuous infusion of 2.0 µg/min). The PTZ infusion was administered via the jugular vein cannula used for diazepam infusion so that the diazepam infusion was terminated at the time the PTZ infusion was begun. The diazepam plasma concentration is expected to drop slightly during the time that the PTZ infusion test was conducted. We therefore corrected the threshold values using a correction factor $e^{-t \ln \left( \frac{2}{t_1/2} \right)}$, where $t$

**FIGURE 1** Plasma levels of diazepam and its metabolites nordiazepam, temazepam, and oxazepam after an acute intravenous bolus injection of diazepam (80 µg/kg). Blood samples were collected 5, 10, 30, 60, 90, 120, and 240 minutes after the diazepam bolus. Data points represent mean ± SEM of measurements in 8 animals. The levels of nordiazepam and temazepam were below the limit of detection in all animals at 90 and 240 minutes, respectively, and therefore no data values are shown at or after these time points.

**TABLE 1** Individual animal noncompartmental parent pharmacokinetic parameters after a bolus intravenous injection of diazepam (80 µg/kg)

<table>
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<tr>
<th>Rat number</th>
<th>Body weight (g)</th>
<th>$C_1$ (ng/mL)</th>
<th>AUC$_{0-240}$ (min ng/mL)</th>
<th>$t_{1/2a}$ (min)</th>
<th>$t_{1/2b}$ (min)</th>
<th>$V_d$ (mL)</th>
<th>$V_d$ (mL/kg)</th>
<th>CL (mL/min)</th>
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<td>1000</td>
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Mean ± SEM 360 ± 3 41.3 ± 2.8 1372 ± 122 13.7 ± 2.1 76.8 ± 12.6 608.4 ± 74.1 1692.7 ± 211.7 22.14 ± 1.86

AUC$_{0-240}$, area under the curve from time of bolus injection to the time of last sample (240 min); $C_1$, plasma concentration at first measurement after bolus injection (5 min); $t_{1/2a}$, initial or disposition half-life; $t_{1/2b}$, elimination half-life; $V_d$, volume of distribution; CL, clearance.
is the time of onset of clonus after initiation of PTZ infusion. Correcting for the drop-off in concentration during
the on-average 10.4 and 5.7 minutes between onset of PTZ infusion (and termination of diazepam infusion) and seizure
sign (clonus) responses for the 4.5 and 2 \( \mu \)g/min PTZ infusion,
respectively, the estimated concentration of diazepam
that is associated with elevation in the seizure sign
threshold is approximately 118 ng/mL, whereas an estimated
concentration of 50 ng/mL does not elevate any seizure
sign.

3.3 | Effect of loading dose and continuous intravenous infusion of diazepam on diazepam
and metabolite levels and seizure sign thresholds

The continuous intravenous infusion paradigm requires a
relatively long period of infusion, which may lead to toler-
ance, a well-known liability of benzodiazepines (Loscher
and Schwark22). To reduce the duration of exposure to dia-
zepam prior to seizure testing, we administered a bolus
dose of diazepam to rapidly bring the diazepam level to
the desired steady-state value followed by continuous infu-
sion to maintain the target level. The maintenance doses
(MDs) were chosen to target \( C_{ss} \) values spanning the range
demonstrated in the previously described continuous infusion
experiments to be at a level below that associated with
activity to a level that is strongly active. The latter was
arbitrarily set at 3.5 \( \mu \)g/min, which was expected based on
the experiment of Figure 2, to achieve a strongly active
plasma level. Additional continuous infusion rates were set
at approximately 40% (1.4 \( \mu \)g/min) and 60% (2.0 \( \mu \)g/min)

of this value. The loading doses were chosen to overshoot

\[ LD = C_{ss} \times V_d \]

the target based on the formula \( LD (\mu g) = C_{ss} \times V_d \)

(608.4 mL), where LD is the loading dose. Thus, to
achieve peak target concentrations of 60, 90, and 150 ng/

mL, loading doses of 36.5, 54.8, and 91.4 \( \mu \)g were admin-
istered.
Using the dosing schemes LD 91.4 μg MD 3.5 μg/min; LD 54.8 μg MD 2.0 μg/min; and LD 36.5 μg MD 1.4 μg/min, mean plasma levels of 78.3 ± 12.2 ng/mL, 35.0 ± 6.2 ng/mL, and 16.3 ± 2.1 ng/mL were obtained at the end of the 77-minute infusion (Figure 4A). The mean levels of the 3 metabolites were in all cases >10-fold less than that of diazepam (Figure 4B-D).

The intravenous PTZ infusion test was conducted at the end of the 77-minute infusion for rats receiving vehicle or diazepam administered with each of the 3 dosing schemes (Figure 5). Statistically significant elevations at all 3 seizure endpoints were observed only with the high-dose scheme (LD 91.4 μg/min, MD 3.5 μg/min). The intermediate dose scheme (LD 54.8 μg/min, MD 2.0 μg/min) was associated with a statistically significant increase in thresholds for myoclonic jerks and tonus but not clonus. The low-dose scheme (LD 36.5 μg/min, MD 1.4 μg/min) failed to elevate the threshold for any of the seizure endpoints. The uncorrected mean plasma concentrations at the end of the 77-minute infusions for the high, intermediate, and low-dose schemes were 78.3, 35.0, and 16.3 ng/mL, respectively. When we corrected for the drop-off in concentration during the on-average 5.9, 3.9, and 2.7 minutes between onset of PTZ infusion and seizure sign responses in the high, intermediate, and low diazepam dosing scheme groups, the plasma concentration of diazepam that is associated with a clear elevation in the seizure threshold was approximately 74 ng/mL (high dosing scheme), whereas an estimated concentration of 34 ng/mL (intermediate dosing scheme) is equivocal and an estimated concentration of 16 (low dosing scheme) was not associated with a threshold alteration for any seizure sign.

4 | DISCUSSION

This study for the first time has provided an estimate of the minimum plasma level of diazepam under near–steady-state conditions that elevates the seizure threshold. To assay seizure threshold, we used the timed intravenous PTZ infusion test, a seizure model that is highly sensitive to diazepam and readily allows quantification of diazepam effects on seizure threshold.23 To achieve the near–steady-state condition, diazepam was administered by continuous intravenous infusion at different doses without and with an initial loading dose. Diazepam was infused through a permanently implanted jugular vein cannula, and blood for determination of diazepam and metabolites was withdrawn from a permanently implanted catheter in the contralateral jugular vein. In the initial set of experiments, a highly
followed by Tukey **** 26.49 myoclonic jerk, clonus, and tonic extension, respectively, were the vehicle-treated groups (V), which (in milligrams per kilogram) for values from 6 to 8 rats normalized with respect to the thresholds in intravenous PTZ infusion in rats. PTZ threshold was measured at the diazepam at different loading and maintenance doses on myoclonic concentrations than previously recognized, the human clinical antiseizure activity occurs at lower steady-state plasma con-

substantial uncertainty. Although our results indicate that seizures in humans (see Introduction). However, the litera-
tion of diazepam is approved for the treatment of status
epilepticus and severe recurrent convulsive seizures.4,31 A
other study, Löscher and Schwark 22 obtained a 
values for \( t_{1/2a} \) and \( t_{1/2b} \) of, respectively, 0.23 and 1.3 hours, are in good agreement with the values reported previously by Klotz et al 30 of 0.29 and 1.1 hours. In another study, Löscher and Schwark 22 obtained a \( t_{1/2b} \) value of 1.4 hours. Klotz et al 30 obtained a plasma clearance of 24.5 mL/min, which is similar to the clearance obtained in the present study of 22.14 mL/min.

Currently, in the United States, an intravenous formulation of diazepam is approved for the treatment of status epilepticus and severe recurrent convulsive seizures.4,31 A rectal gel formulation (Diastat) is approved for the outpatient treatment of bouts of increased seizure activity (seizure clusters).32 The rectal formulation of diazepam is limited by poor patient and caregiver acceptance. Rectal formulations also often exhibit erratic and unpredictable relevance of our findings is uncertain. We further note that the potency of diazepam varies in different seizure models and that the potency against PTZ-induced seizures is particularly robust.2 With these caveats, our results suggest that plasma levels of diazepam in the range of 70 ng/mL and possibly even lower could have clinical activity.

Diazepam is well recognized to undergo demethylation and oxidative metabolism to active metabolites. The principal metabolite is nordiazepam, which is roughly 4-fold less potent than diazepam as a modulator of GABAA receptors and as an antiseizure agent in rats.24,25 However, levels of nordiazepam in our experiments were only a fraction of that of diazepam, even after sufficient time had elapsed to nearly achieve steady-state. This observation is consistent with prior studies showing little accumulation of nordiazepam in cultured rat hepatocytes and in rats in vivo.12,26,27 Overall, nordiazepam is expected to have contributed negligibly to the antiseizure effects observed in the present study. Nordiazepam is metabolized to oxazepam, which is also active as an anticonvulsant but is substantially less potent than diazepam.28,29 Diazepam is additionally metabolized to temazepam (N-methyl oxaze-
pam), which is also metabolized to oxazepam. However, levels of temazepam and oxazepam were even lower than that of nordiazepam and would therefore have even less influence on the outcome. There is greater production of the active metabolites in humans.17,19 Therefore, the threshold diazepam concentration determined here in rats may overestimate to some extent the level required to confer seizure protection in humans, particularly with dosing schemes that allow the accumulation of substantial levels of active metabolites.

The pharmacokinetic parameters obtained in our study with bolus dosing are quantitatively similar to values reported in the literature. Following acute administration of diazepam, blood levels of the parent are well described by a 2-compartment model with a rapid distribution phase (\( \alpha \)) followed by a longer elimination phase (\( \beta \)).30 Our half-life values for \( t_{1/2a} \) and \( t_{1/2b} \) of, respectively, 0.23 and 1.3 hours, are in good agreement with the values reported previously by Klotz et al30 of 0.29 and 1.1 hours. In another study, Löscher and Schwark22 obtained a \( t_{1/2b} \) value of 1.4 hours. Klotz et al30 obtained a plasma clearance of 24.5 mL/min, which is similar to the clearance obtained in the present study of 22.14 mL/min.

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absorption. Alternative routes of diazepam administration, including intranasal, buccal, or intramuscular, may be preferred.\textsuperscript{3,33,34} Nasal spray formulations that administer diazepam doses of 4-20 mg have been reported to result in mean diazepam blood levels of approximately 100-400 ng/mL.\textsuperscript{33,35} Our study confirms that these exposures are associated with relevant pharmacodynamic activity, and, indeed, modestly lower exposures may be sufficient to confer seizure protection, which may avoid adverse effects including oversedation, hypotension, and respiratory depression that can occur with high diazepam doses.

ACKNOWLEDGMENTS
This research was supported by the CounterACT Program, National Institutes of Health Office of the Director, and the National Institute of Neurological Disorders and Stroke under Grant NS079202, and by Acorda Therapeutics.

DISCLOSURE
The authors declare no competing financial interests. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID
Michael A. Rogawski \textsuperscript{a} http://orcid.org/0000-0002-3296-8193

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How to cite this article: Dhir A, Rogawski MA. Determination of minimal steady-state plasma level of diazepam causing seizure threshold elevation in rats. Epilepsia. 2018;00:1–10. https://doi.org/10.1111/epi.14069