Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 465 (2003) 257–265
www.elsevier.com/locate/ejphar

Evaluation of the novel antiepileptic drug, AWD 131-138, for benzodiazepine-like discriminative stimulus and reinforcing effects in squirrel monkeys

Sevil Yasar[a,b,*], Jack Bergman[c], Patrik Munzar[b], Godfrey Redhi[b], Christine Tober[d,1], Norbert Knebel[e], Michael Zschiesche[f], Carol Paronis[c]

[a] Division of Geriatric Medicine and Gerontology, Johns Hopkins University School of Medicine, Baltimore, MD, USA
[b] Behavioral Neurosciences Branch, National Institute on Drug Abuse, Intramural Research Program, National Institutes of Health, Department of Health and Human Services, Baltimore, MD, USA
[c] Department of Psychiatry, Harvard Medical School, Boston, MA, USA
[d] Department of Pharmacology, Arzneimittelwerk Dresden GmbH, Radebeul, Germany
[e] Department of Pharmacokinetics and Bioanalytics, ASTA Medica AG, Frankfurt/Main, Germany
[f] Institute of Pharmacology, Ernst-Moritz-Arndt University, Greifswald, Germany

Received 14 February 2003; accepted 21 February 2003

Abstract

AWD 131-138 {1-(4-chlorophenyl)-4-morpholino-imidazolin-2-one}, a new low-affinity partial benzodiazepine receptor agonist with potent anticonvulsant and anxiolytic properties in rodent models, was studied in squirrel monkeys trained to discriminate intramuscular (i.m.) injections of midazolam (0.3 mg/kg) from injections of vehicle. Diazepam produced midazolam-like responding at cumulative doses of 1.0 and 3.0 mg/kg i.m. and decreased rates of responding at 3.0 mg/kg (plasma levels of about 400 ng/ml). In contrast, AWD 131-138 did not produce midazolam-like responding or alter response rates at cumulative doses up to 18.0 mg/kg i.m. (plasma levels over 2100 ng/ml). Other monkeys were trained to intravenously (i.v.) self-administer cocaine (56.0 μg/kg/injection). When AWD 131-138 (10–100 μg/kg/injection) was studied by substitution, responding declined to vehicle substitution levels within three sessions. At the dose of 100 μg/kg i.v. AWD 131-138, sufficient drug was self-administered during the first session (about 3.5 mg/kg) to produce plasma levels above 1000 ng/ml, yet responding over the next two sessions dropped to vehicle levels. The failure of AWD 131-138 to produce benzodiazepine-like discriminative effects and the absence of drug self-administration behavior when substituted for cocaine suggest that its abuse liability is low.

Keywords: Anticonvulsant; Benzodiazepine; AWD 131-138; Midazolam; Diazepam; Drug discrimination; Self-administration, monkey

1. Introduction

Drugs currently available for the treatment of anxiety and epilepsy have a variety of side effects including drowsiness, mental dullness, nausea, ataxia, paresthesia, hematologic changes, and weight gain. AWD 131-138 {1-(4-chlorophenyl)-4-morpholino-imidazolin-2-one} is a new chemical entity (Heinecke and Thiel, 2001; Fig. 1) currently in phase II clinical development as an anxiolytic and anticonvulsant (Jain, 2000). It was selected for development because it has a broad spectrum of anticonvulsant activity and is active in rodent models predictive of anxiolytic effects (Walker and Sander, 1998; Rostock et al., 1998b).

In mouse, rat, and dog seizure models, AWD 131-138 has anticonvulsant activity at doses clearly below those producing motor impairment (Bialer et al., 1999, 2001; Tober et al., 1998, 1999, 2000). For example, the ED_{50} values in rats for AWD 131-138 after oral dosing are 13 mg/kg in the maximal electroshock test and 27 mg/kg in the pentylenetetrazol test (Rostock et al., 1998b). In contrast, in the rotorod test, which is used to preclinically assess

* Corresponding author. Johns Hopkins Bayview Medical Center, 5505 Hopkins Bayview Circle, Baltimore, MD 21224, USA. Fax: +1-410-550-0182, +1-410-769-8758 (home).
E-mail address: syasar@jhmi.edu (S. Yasar).
[1] Current address: Marketing Service, AWD. Pharma GmbH and Co. KG, Dresden, Germany.

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01533-4

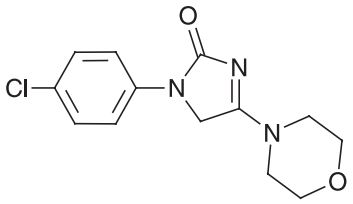

Fig. 1. Chemical structure of AWD 131-138. AWD 131-138 is 1-(4-chlorophenyl)-4-morpholino-imidazolin-2-one.

minimal neurotoxicity, it has an oral ED_{50} of 998 mg/kg (Bialer et al., 1999, 2001). In addition, AWD 131-138 is active at oral or intraperitoneal (i.p.) doses as low as 3 mg/kg in a variety of mouse and rat models predictive of anxiolytic activity, such as the elevated plus maze and the Vogel drinking conflict test (Rostock et al., 1998a). Thus, AWD 131-138 appears to have a unique pharmacological profile in various in vivo and in vitro preclinical tests, which makes it a promising candidate for clinical trials.

The neuropharmacological mechanisms underlying the anticonvulsant and anxiolytic actions of AWD 131-138 in preclinical tests are not fully clear. AWD 131-138 binds to a specific allosteric site on type A gamma aminobutyric acid receptors ($GABA_A$ receptors, the benzodiazepine binding site), although with very low affinity relative to other benzodiazepines (IC_{50} of about 5.8 μM; Rostock et al., 1998b; Sigel et al., 1998). The intrinsic activity was indicative of a partial agonistic interaction. At 30 μM, only 12–21% of the maximal GABA-potentiating effect obtained with diazepam was reached (Sigel et al., 1998). In addition, AWD 131-138 (1 and 100 μM) antagonizes the increased firing of action potentials induced by corticotrophin-releasing factor in locus coeruleus neurons of murine brainstem slices, but has no effects on the normal frequency of action potential firing in these neurons (Rundfeldt et al., 1999). A similar effect could be obtained with diazepam in this preparation, supporting the view that AWD 131-138 acts as a (partial) benzodiazepine agonist (Rundfeldt, personal communication, 2001). In addition, AWD 131-138 also blocks voltage-activated calcium channels in a dose-related manner. A significant block is found at concentrations as low as 1 μM and it is, as yet, unclear which channel subtype is affected. In contrast, when tested for its interaction with more than 40 other receptors, ion channels, or second-messenger systems, AWD 131-138 does not inhibit binding or activity (Rostock et al., 1998b).

Since AWD 131-138 binds to the benzodiazepine binding site on the $GABA_A$ receptor, although weakly, and, like a variety of clinically used benzodiazepines, appears to have both anxiolytic and anticonvulsant activity in preclinical tests in rodents, it was of interest to evaluate it for potential benzodiazepine-like abuse liability. In the present study, potential benzodiazepine-like abuse liability of AWD 131-138 was evaluated using a two-lever drug discrimination procedure and an intravenous (i.v.) drug self-administration procedure in squirrel monkeys.

Drug discrimination procedures are frequently used to preclinically evaluate benzodiazepine-like abuse liability of drugs by testing the efficacy and potency of a drug in substituting for a prototype benzodiazepine in experimental animals trained to discriminate injections of benzodiazepine from injections of vehicle. Drugs acting in the central nervous system can induce changes in interoceptive states that serve as discriminative stimuli in animals and, for diverse classes of drugs including benzodiazepines, there is pharmacological specificity in the classification of drugs based on their generalized discriminative stimulus effects (e.g., Colpaert et al., 1976; Garcha et al., 1985; Sanger and Benavides, 1993). In the present experiment, AWD 131-138 and, for comparison, diazepam were tested by substitution in squirrel monkeys trained to discriminate injections of the short-acting benzodiazepine, midazolam, from injections of vehicle.

A second procedure frequently used to preclinically evaluate a compound's abuse liability is to test the compound's efficacy and potency in maintaining self-administration behavior in monkeys trained to i.v. self-administer a prototype drug of abuse, usually cocaine (e.g., Balster, 1991). Most drugs that are abused by humans are self-administered by monkeys (e.g., Johanson and Balster, 1978; Young and Herling, 1986; Woolverton and Nader, 1990), including benzodiazepines (e.g., Katz et al., 1991; Griffiths and Weerts, 1997; Munzar et al., 2001). For example, in baboons trained to i.v. self-administer cocaine, a series of six benzodiazepines, including diazepam, flurazepam, and midazolam, maintained self-administration behavior above vehicle levels when directly substituted for cocaine (Griffiths et al., 1981, 1991). In the present experiment, AWD 131-138 was tested by substitution in squirrel monkeys trained to intravenously self-administer cocaine in order to determine whether or not it maintains significant self-administration behavior.

2. Materials and methods

2.1. Subjects

Thirteen adult male squirrel monkeys (*Saimiri sciureus*) weighing 0.7–1.1 kg were maintained on a 12-h light/dark cycle (lights on from 6:45 a.m. to 6:45 p.m.). Four monkeys were used in a drug discrimination study, three in a drug self-administration study, and six for determining plasma levels of tested substances. Daily experimental sessions (5–7 days/week) were conducted during the light phase. Between sessions, the monkeys were housed in individual cages in a climate-controlled vivarium with unrestricted access to water and food (PMI High-Protein Diet and daily fresh fruit). All monkeys had previous experience with benzodiazepines or other psychoactive drugs.

The three monkeys used in the self-administration study were each surgically prepared with a chronic i.v. polyvinyl

chloride catheter (inside diameter, 0.38 mm; outside diameter, 0.76 mm). The catheter was passed through the right or left external or internal jugular vein, or right or left external iliac vein to the level of the right atrium under halothane anesthesia. Catheters led subcutaneously to the monkey's back where they exited the skin. The monkeys wore leather jackets at all times to protect the catheters. Each weekday, the catheters were flushed with saline, refilled with saline, and sealed with stainless steel obturators. At the start of the experiments, two monkeys had a history of cocaine self-administration and the third had a history of both cocaine and nicotine self-administration, but they had not been previously exposed to either barbiturates or benzodiazepines.

Animals used in this study were maintained in facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all experimentations were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Intramural Research Program, National Institute on Drug Abuse, NIH, and the Guide for Care and Use of Laboratory Animals (National Research Council, 1996).

2.2. Apparatus

A Plexiglas chair was enclosed in a sound-attenuating isolation chamber with white noise for masking of external sound. The transparent front panel of the chair contained one response lever (self-administration study) or two levers spaced 12 cm apart (drug discrimination study) (no. 121-05; BRS/LVE, Laurel, MD); each press of a lever with a force greater than 0.2 N produced an audible click and was recorded as a response. Pairs of colored stimulus lights, mounted behind the transparent front panel of the chair, could be illuminated and used as visual stimuli. In the drug discrimination study, a shaved portion of each monkey's tail was coated with electrode paste before each session and placed under brass electrodes for delivery of brief, low-intensity electric shock stimuli (200 ms, 3.0 mA). In the drug self-administration study, before each session, the monkey's catheter was connected to polyethylene tubing, which passed out of the isolation chamber where it was attached to a motor-driven syringe pump (model No. 57-6496; Harvard Apparatus, South Natick, MA). Equipment was controlled by computers using basic programming (drug discrimination study) or (self-administration study) a MED Associates MED-PC software package (MED Associates, East Fairfield, VT).

2.3. Drug discrimination procedure

Monkeys were trained, as reported previously (e.g., Yasar and Bergman, 1994; Tidey and Bergman, 1998), to respond under a fixed ratio 10 (FR10) schedule of stimulus–shock termination. Under this schedule, the completion of 10 responses on one of two levers terminated red stimulus lights associated with shock delivery every 20 s. Either completion of the FR or the delivery of four shocks initiated a 30-s timeout period, during which the chamber was dark and responding had no programmed consequences.

Once responding was stable under the FR10 schedule, monkeys were trained to discriminate intramuscular (i.m.) injections of 0.3 mg/kg midazolam from saline. After midazolam injection, 10 consecutive responses on one lever terminated the stimulus lights and the programmed shock delivery. After saline administration, 10 responses on the other lever terminated the lights and the programmed shock delivery. The left lever was associated with midazolam injection in two monkeys, and the right lever was associated with midazolam injection in the remaining two monkeys. During all training sessions, responses on the incorrect lever reset the response requirement. The session ended after 10 presentations of the FR schedule.

When discrimination performance was stable, daily training sessions were expanded to comprise one to four components, each component starting with a 10-min timeout period followed by 10 presentations of the FR10 schedule. During training sessions, saline or midazolam was administered at the start of each 10-min timeout period. The number of components in daily training sessions varied on a random basis with the provisos that (1) midazolam was injected only before the last component of the session, and (2) sessions with injections only of saline occurred periodically to avoid invariant association between injection of midazolam and the last session component.

Drug effects were determined during test sessions conducted once or twice per week, and training sessions were conducted on intervening days. During test sessions, the effects of midazolam (0.03–1.0 mg/kg), diazepam (0.1–3.0 mg/kg), and AWD 131-138 (3.0–18.0 mg/kg) were studied using a cumulative dosing procedure (cf., Yasar and Bergman, 1994; Tidey and Bergman, 1998). Testing of higher doses of AWD 131-138 was precluded by solubility problems. With the cumulative dosing procedure, drugs were administered at the start of each 10-min timeout period such that the total dose increased by 0.25 or 0.5 $\log_{10}$ unit increments throughout the session (e.g., 3.0 mg/kg AWD131-138 at the start of the first timeout, 7.0 mg/kg at the start of the second timeout, and 8.0 mg/kg at the start of the third timeout for a cumulative dose of 18.0 mg/kg).

2.4. Self-administration procedure

Before the start of each session, monkeys were placed into the Plexiglas chairs and restrained in the seated position by waist locks. Their i.v. catheters were connected to motor-driven syringes as described above. Monkeys were trained, as reported previously (Goldberg, 1973; Spear et al., 1991; Tanda et al. 2000), to self-administer 56.0 μg/kg injections of cocaine. At the start of each session, one i.v. injection was delivered to fill the ''dead volume'' of the catheter, the white

house light was turned off, and green stimulus lights were illuminated. In the presence of the green lights, monkeys were required to make 10 responses on the lever (10-response, fixed-ratio schedule of reinforcement; FR10). The completion of 10 responses on the lever turned off the green lights and produced an i.v. injection of cocaine or saline placebo paired with a 2-s illumination of amber stimulus lights. Injection duration was 0.2 s and volume of injection was 0.2 ml. Each injection was followed by a 60-s timeout period, during which the chamber was dark and lever presses had no programmed consequences. Each session lasted for 1 h and sessions were conducted 5–6 days/week.

When responding was stable under the FR10 schedule of cocaine injection, different doses of AWD 131-138 (10.0, 30, or 100.0 μg/kg/injection) or vehicle (either saline or 15% Emulphor) were substituted for cocaine. Each dose of AWD 131-138 or vehicle was substituted for three sessions, and responding was restabilized on cocaine self-administration before testing the next dose of AWD 131-138. Further testing of higher doses of AWD 131-138 was precluded by solubility problems and a tendency for the Emulphor vehicle to clog the i.v. catheters.

2.5. Measurement of plasma levels of AWD 131-138, diazepam, and nordiazepam

Separate groups of monkeys were maintained on a non-restricted diet and received i.m injections of AWD 131-138 or diazepam using the same cumulative dosing procedure used in the drug discrimination experiments. That is, consecutive i.m. injections of 3, 7, and 8 mg/kg AWD 131-138 (cumulative dose of 18 mg/kg) or 0.1, 0.2, 0.7, and 2.0 mg/kg diazepam (cumulative dose of 3 mg/kg) were administered with an interinjection interval of 17 min. Plasma samples were then taken 5, 13, and 25 min after the last i.m. injection. To parallel the self-administration experiments, in which an average of 35 injections of AWD 131-138 were taken at the high 100 μg/kg injection dose during the first substitution session, a separate group of catheterized monkeys was given 35 consecutive i.v. injections of 100 μg/kg AWD 131-138 with an interinjection interval of 60 s (cumulative dose of 3.5 mg/kg) . Plasma samples were then taken 1, 5, and 25 min after the last i.v. injection. All plasma samples were stored at −20 °C up to the time of analysis.

Plasma levels of AWD 131-138, diazepam, and its active metabolite nordiazepam were performed at Asta Medica (Frankfurt, Germany) and the University of Greifswald (Greifswald, Germany) according to established high-pressure liquid chromatography (HPLC) methods. Briefly, AWD 131-138 was analyzed using a validated HPLC mass spectrometry (HPLC/MS) assay. The plasma sample work-up was performed by protein precipitation with acetonitrile. The chromatography was performed on a Luna (Phenomenex®; 5 μm, 100 Å, 50 × 4.6 mm) analytical column at a flow rate of 0.5 ml/min. Mass spectrometry was carried out using a PE Sciex (API 365/3000) in the selected reaction monitoring (SRM) mode with the APCI interface. The mass spectrometer operated in the positive ion mode using 80% acetonitrile/20% 0.0025 M ammonium acetate (pH 6.5) (vol/vol). The selected precursor ions and product ions for AWD 131-138 were at *m*/*z* 280 and *m*/*z* 237, respectively. The lower limit of quantification for AWD 131-138 was determined to be 5.0 ng/ml, with a limit of detection of 2.0 ng/ml. The standard curve was linear from 5.0 to 5000 ng/ml using weighted linear regression analysis ($1/y^2$) (response-squared weighting).

For analysis of diazepam and nordiazepam, an available HPLC method for human plasma analysis was validated. Briefly, 50 μl of plasma was mixed with sodium borate buffer and an internal standard (clobazam). Then, the mixture was double-extracted with ether. The evaporated ether phase was dissolved in a 100-μl mobile phase and

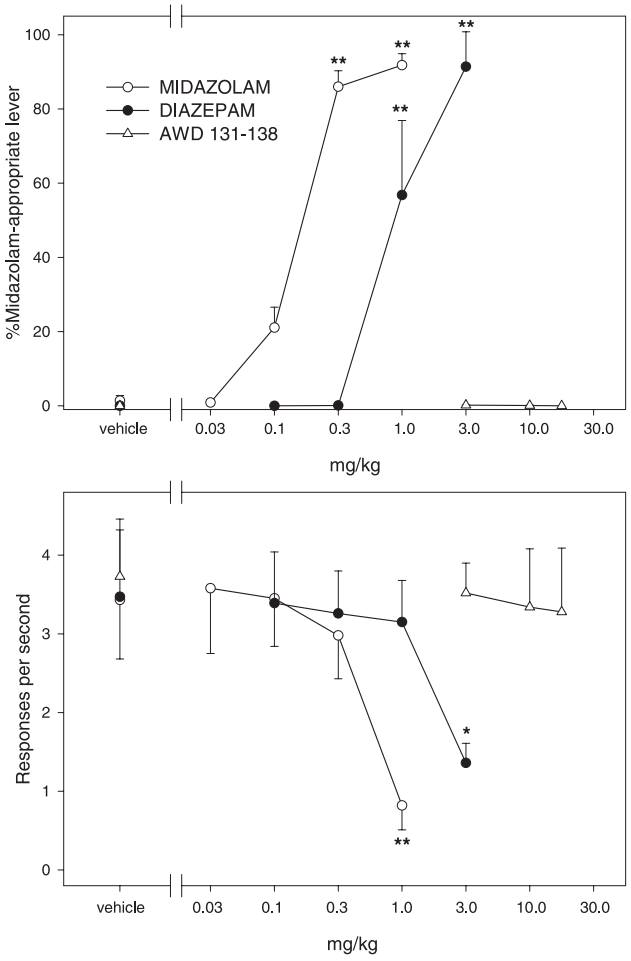

Fig. 2. Drug discrimination study. Effects of i.m. doses of midazolam (○), diazepam (●), and AWD 131-138 (△) in squirrel monkeys trained to discriminate midazolam (0.3 mg/kg i.m.) from vehicle. Data are means (± S.E.M.) from three to four subjects. The percentage of midazolam-appropriate responding (upper panel) and running rates of responding in responses per second (lower panel) are shown as functions of dose during substitution test sessions. *$P < 0.05$, **$P < 0.01$, post-hoc comparisons with vehicle conditions after significant one-way ANOVA for repeated measures main effect; Dunnett's test.

Table 1
Plasma levels of AWD 131-138, diazepam, and nordiazepam in squirrel monkeys

Time after injection (min)	Injection route	*n*	AWD 131-138 (ng/ml)	Diazepam (ng/ml)	Nordiazepam (ng/ml)
5	i.m.	3	2162 ± 389	401 ± 216	33 ± 10
13	i.m.	3	2174 ± 690	435 ± 177	78 ± 18
25	i.m.	3	2685 ± 464	332 ± 94	103 ± 20
1	i.v.	3	822 ± 709		
5	i.v.	3	849 ± 424		
25	i.v.	3	1041 ± 639		

Shown are mean ± S.D. (ng/ml) of plasma levels 5, 13, and 25 min after a cumulative intramuscular dose of either 18.0 mg/kg AWD 131-138 or 3.0 mg/kg diazepam, and 1, 5, and 25 min after 35 successive intravenous injections of 100 μg/kg AWD 131-138 (a cumulative i.v. dose of 3.5 mg/kg).

analyzed in an isocratic reverse-phase HPLC system (244 nm UV). The limit of detection and lower limit of quantification were determined at 5 and 12.5 ng/ml for both analyses. The calibration curves were linear between 12.5 and 1500 ng/ml plasma, evaluated with $1/x$ weighting of the regression analysis (x = concentration).

2.6. Data analysis

All results are presented as group mean ± S.E.M. In the drug discrimination study, response rates were calculated as run rates (i.e., the total number or responses in each component was divided by the duration of the component minus the sum of latencies to the first lever press in each presentation of the FR10 schedule and minus the sum of all 30-s timeout periods). Percent drug lever responding was calculated by dividing the number of responses on the midazolam-associated lever by the total number of responses on both levers. Percent drug lever responding was not calculated for components in which response rates were less than 0.2 responses/s. Full substitution with a test drug was considered to have occurred when drug lever responding was 90%. In the self-administration study, results are expressed as total number of injections per 1-h session and as responses per second averaged over the session, with responding during timeouts not included in calculations.

Statistical analysis was done using one-way analysis of variance (ANOVA) for repeated measures. Significant main effects were analyzed further by subsequent paired comparisons to control values using a post-hoc Dunnett's test. Vehicle conditions served as a control in the drug discrimination study. For constructing the dose–response curve in the self-administration study, means from the last two sessions under each condition were used and were compared with vehicle self-administration levels. The last session of cocaine self-administration prior to substitution served as a control in analysis of consecutive sessions during substitution tests. Changes were considered to be significant when $P < 0.05$.

2.7. Drugs

In the drug discrimination study, midazolam maleate and diazepam (both donated by Hoffmann-La Roche, Nutley, NJ) were initially dissolved in a vehicle of 20% ethanol, 20% Alkamus EL-620 (Emulphor; Rhone-Poulenc, Cranbury, NJ), and 60% saline, and were diluted in saline. AWD 131-138 (Arzneimittelwerk Dresden GMBH, Radebeul, Germany) in doses of 3.0–18.0 mg/kg was dissolved in 100% DMSO and diluted with Emulphor and sterile water such that a final solution of 10 mg/ml AWD 131-138 contained 10% DMSO, 45% Emulphor, and 45% water.

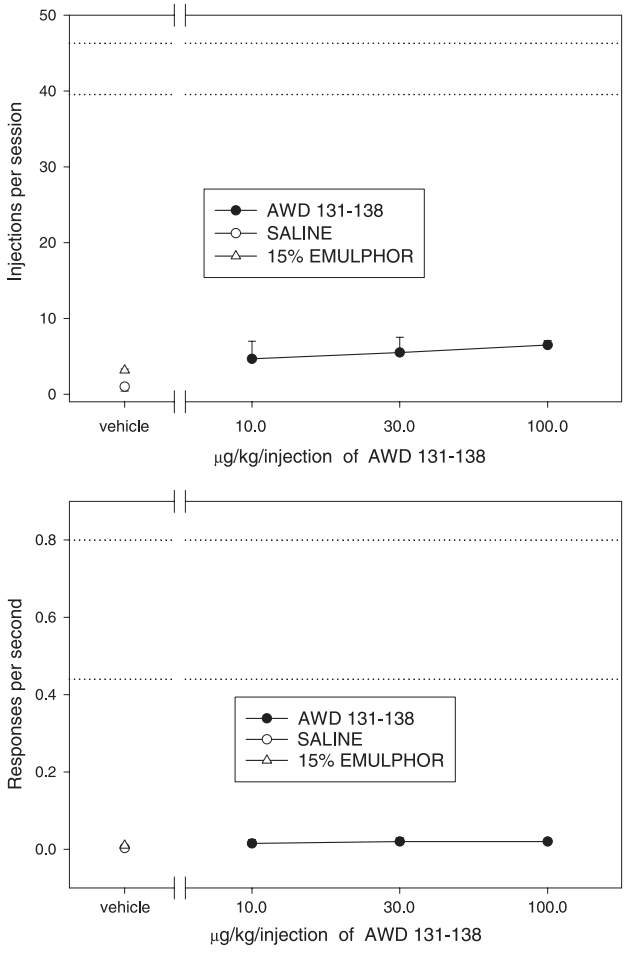

Fig. 3. Self-administration study. AWD 131-138 dose–response curve in squirrel monkeys trained to intravenously self-administer 56.0 μg/kg/injection cocaine. Total number of injections (upper panel) and rates of responding (lower panel) are presented as functions of AWD 131-138 dose. The area between the dotted lines represents mean values (± S.E.M.) for number of injections and response rates during the two cocaine self-administration sessions (56.0 μg/kg/injection) preceding substitution of each dose of AWD 131-138 or vehicle. Each point represents the mean from the last two sessions under each vehicle or AWD 131-138 dosage condition. Data are means (± S.E.M.) from three subjects. One-way ANOVA for repeated measures revealed that there was no significant difference between vehicle conditions and any AWD 131-138 dosage condition.

Injections were given i.m. in volumes of 0.3–1.0 ml/kg of body weight.

In the self-administration study, (−)-cocaine hydrochloride (National Institute on Drug Abuse, Rockville, MD) and AWD 131-138 were dissolved in saline. Injections were given i.v. in volumes of 0.2 ml per injection over a 200-ms duration. At the highest dose tested (100 μg/kg/injection), AWD 131-138 was dissolved in Emulphor and diluted in saline such that the final solution of AWD 131-138 contained 15% Emulphor and 85% saline.

Doses of midazolam, diazepam, and AWD 131-138 refer to the weight of the free drug, whereas the dose of cocaine refers to the weight of the salt.

3. Results

In the drug discrimination study, midazolam (0.03–1.0 mg/kg i.m.) produced dose-dependent increases in responding on the midazolam-appropriate lever [$F(4,11) = 144.24$, $P < 0.001$; one-way ANOVA for repeated measures] with full generalization after the 0.3 mg/kg training dose of midazolam and a higher dose of 1.0 mg/kg. Diazepam (0.1–3.0 mg/kg i.m.) also produced dose-related increases in midazolam-appropriate responding [$F(4,12) = 22.21$, $P < 0.001$] with full generalization after a dose of 3.0 mg/kg diazepam. The highest tested doses of midazolam [1.0 mg/kg; $F(4,12) = 5.53$, $P = 0.009$] and diazepam [3.0 mg/kg; $F(4,12) = 4.34$, $P = 0.021$] significantly decreased rates of responding. In contrast, AWD 131-138 (3.0–18.0 mg/kg i.m.) did not produce midazolam-appropriate responding in any monkey tested ($P > 0.05$), did not alter rates of responding ($P > 0.05$), and did not produce any observable changes in gross behavior (Fig. 2).

Plasma levels of AWD 131-138, diazepam, and its active metabolite nordiazepam, which were obtained using the cumulative dosing procedure employed in the drug discrimination study, are shown in Table 1. Plasma levels of AWD 131-138 after the cumulative i.m. dose of 18 mg/kg, which did not produce midazolam-like discriminative stimulus effects, were over fivefold higher than the combined plasma levels of both diazepam and its active metabolite, nordiazepam, after the cumulative i.m. dose of 3 mg/kg of diazepam, which completely substituted for the discriminative stimulus effects of the training dose of midazolam.

In the self-administration study, a 56.0-μg/kg/injection dose of cocaine maintained high average response rates (averaging 0.62 ± 0.18 response/s) and a stable level of injections per 1-h session (averaging 42.9 ± 3.38). In contrast, only low rates of responding were observed for i.v. injections of vehicle (saline or 15% Emulphor) or 10.0–100.0 μg/kg/injection doses of AWD 131-138. One-way ANOVA for repeated measures furthermore revealed that there was no significant difference between vehicle self-administration and self-administration of any dose of AWD 131-138 tested when both numbers of injections ($P > 0.05$) and rates of responding ($P > 0.05$) were analyzed (Fig. 3). During substitution of saline [$F(3,6) = 257.47$, $P < 0.001$], 15% Emulphor [$F(3,6) = 20.39$, $P = 0.002$], 10.0 μg/kg/injection of AWD 131-138 [$F(3,6) = 74.66$, $P < 0.001$], or 30.0 μg/

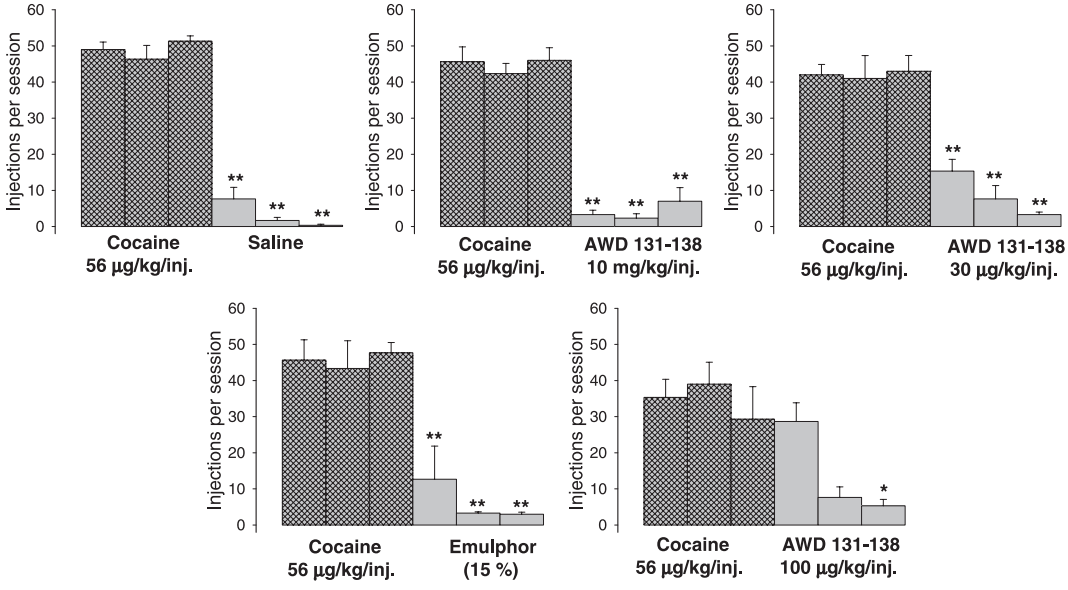

Fig. 4. Self-administration study. Effects of different i.v. doses of AWD 131-138 and vehicle (saline for the 10.0- and 30.0-μg/kg/injection doses of AWD 131-138, and 15% Emulphor for the 100.0-μg/kg/injection dose of AWD 131-138) in squirrel monkeys trained to intravenously self-administer 56.0 μg/kg/injection of cocaine. Cross-hatched bars represent responding during three consecutive cocaine sessions immediately preceding substitution sessions. Open bars represent responding during three consecutive substitution sessions when different vehicles and different doses of AWD 131-138 were tested. Results are expressed in injections per 1-h session. Data are means (± S.E.M.) from three subjects. *$P < 0.05$, **$P < 0.01$, post-hoc comparisons with the last cocaine self-administration session prior to substitution after significant one-way ANOVA for repeated measures main effect; Dunnett's test.

kg/injection of AWD 131-138 [$F(3,6) = 26.47$, $P < 0.001$] for cocaine, self-administration responding extinguished fully during the first substitution session (Fig. 4) and was significantly different from the last session of cocaine self-administration under all conditions, as revealed by one-way ANOVA for repeated measures. In contrast, during substitution of the 100-μg/kg/injection dose of AWD 131-138 for cocaine, monkeys self-administered AWD 131-138 during the first day of substitution, but responding decreased by the second day and was at vehicle levels on the third day of substitution (Fig. 4). During the first session at the injection dose of 100.0 μg/kg, total i.v. intake of AWD 131-138 ranged from 2.4 to 3.9 mg/kg. As shown in Table 1, plasma levels of AWD 131-138, 1, 5, and 25 min after the last of 35 consecutive i.v. injections of 100 μg/kg AWD 131-138, were 822, 849, and 1041 ng/ml, respectively.

4. Discussion

In the present experiments, AWD 131-138 was inactive in the two behavioral tests in monkeys frequently used to predict benzodiazepine-like abuse liability, substitution for the benzodiazepine discriminative stimulus cue, and maintenance of i.v. self-administration behavior (cf., Griffiths and Weerts, 1997; Garcha et al., 1985). Benzodiazepines clinically used as anticonvulsants, such as diazepam and clonazepam, generally are active in these behavioral tests and also function as full agonists, binding with high affinity to benzodiazepine-binding sites on $GABA_A$ receptors and facilitating GABA-mediated synaptic inhibition. In contrast, AWD 131-138 acts with low affinity and low intrinsic activity as a partial agonist at benzodiazepine-binding sites on the $GABA_A$ receptor (Rostock et al., 1998b; Sigel et al., 1998). For example, the affinity of AWD 131-138 for the benzodiazepine-binding site on the $GABA_A$ receptor ($K_i = 4350$ nM in rat brain) is much lower than the affinity of diazepam ($K_i = 6.8$ nM) or clonazepam ($K_i = 1.7$ nM). Also, AWD 131-138 dose-dependently stimulated GABA-induced chloride currents in five different cloned $GABA_A$ receptor subunit combinations, but this stimulation averaged only 12–21% of the maximal stimulation found with diazepam (Sigel et al., 1998). The low affinity and low intrinsic activity of AWD 131-138 at the benzodiazepine-binding site on the $GABA_A$ receptor suggest that other modes of action may contribute to its effects (Bialer et al., 1999) and may explain its inactivity in the two behavioral tests used to predict benzodiazepine-like abuse liability in the present experiments.

In monkeys and rats trained to discriminate the short-acting benzodiazepine, midazolam, from saline vehicle, classical full-agonist benzodiazepines such as diazepam and chlordiazepoxide will generally fully substitute for the midazolam cue in a dose-dependent manner (e.g., Garcha et al., 1985; Spealman, 1985; Woudenberg and Slangen, 1989; Sannerud and Ator, 1995). In studies by Spealman (1985), for example, squirrel monkeys that were trained to discriminate between injection of 0.3 mg/kg midazolam and saline, using a procedure similar to that employed in the present experiments, showed greater than 90% responding on the midazolam-associated lever when doses of diazepam or chlodiazepoxide that did not severely suppress rates of lever pressing were substituted. Similar results were found with diazepam in the present experiments.

In contrast to the past and present findings with full-agonist benzodiazepines like diazepam, AWD 131-138 did not produce midazolam-like responding in the present drug discrimination study at doses up to 18 mg/kg. In contrast, an 18-fold lower dose of diazepam (1 mg/kg) produced significant midazolam-like discriminative stimulus effects. This is consistent with the findings noted above, that AWD 131-138 exerts only a low affinity for the benzodiazepine-binding site on the $GABA_A$ receptor complex (Rostock et al., 1998b; Bialer et al., 1999, 2001) and that its effects on GABA-induced chloride currents were only about 20% of those of the full agonist, diazepam (Sigel et al., 1998). AWD 131-138 also did not decrease rates of responding in the drug discrimination study, at doses up to 18.0 mg/kg i.m., while a sixfold lower dose of diazepam markedly decreased response rates. This is consistent with the lack of sedative effects of AWD 131-138 in various tests for sedation in rodents. For example, in rats, the minimum effective oral dose of AWD 131-138 in anticonflict and elevated maze tests for anxiolytic activity was 3 mg/kg and its oral ED_{50} dose in the pentylenetetrazol test for anticonvulsant activity was 27.4 mg/kg, while the oral TD_{50} dose for disruption of rotorod performance was 998/mg/kg (Bialer et al., 1999, 2001). In contrast, rotorod performance of rats was disrupted by the oral doses of 1–10 mg/kg diazepam needed to produce anxiolytic and anticonvulsant effects (Rostock et al., 1998a,b). Based on a relative potency of 3:1 of AWD 131-138 to diazepam in tests for anxiolytic and anticonvulsant effects in rats (Rostock et al., 1998a,b; Bialer et al., 1999, 2001), the doses of AWD 131-138 tested in the present experiments should have been sufficiently high.

The lack of benzodiazepine-like discriminative stimulus effects with AWD 131-138 cannot be attributed to poor absorption. Plasma levels of AWD 131-138 after the high i.m. dose of 18 mg/kg (about 2400 ng/ml), which had no midazolam-like discriminative stimulus effects or depressant effects on responding, were over sixfold higher than those of a dose of 3 mg/kg i.m. diazepam (about 400 ng/ml), which completely substituted for the midazolam training stimulus, markedly depressed rates of responding, and were well above plasma levels needed to produce pronounced anticonvulsive activity in rats (800–2100 ng/ml; Tober, unpublished observations) and anticonvulsive effects in dogs (500–1200 ng/ml; Tober, unpublished observations). The lack of effect of AWD 131-138 also cannot be attributed to rapid elimination. Plasma levels of AWD 131-138 continued to increase over the 25-min period of measurements following its i.m. injection. Similarly, AWD 131-138

is well absorbed after oral administration in dogs and rats, with a half-life of approximately 6 h (cf., Bialer et al., 1999).

A large number of studies have been conducted in nonhuman primates indicating that benzodiazepines such as diazepam, clonazepam, and midazolam can serve as reinforcers to maintain intravenous drug self-administration behavior in both drug-naive and drug-experienced animals (e.g., Katz et al., 1991; Griffiths and Weerts, 1997). Benzodiazepines can maintain self-administration behavior of monkeys at rates significantly exceeding vehicle control levels under a number of different access conditions (see review by Griffiths and Weerts, 1997), including those employed in the present experiments (FR schedule with timeouts and 1-h, time-limited access; Munzar et al., 2001). However, benzodiazepines generally maintain lower rates of self-administration than other abused sedative and psychomotor-stimulant drugs, which is consistent with clinical data in humans (Katz et al., 1991; Griffiths and Weerts, 1997).

In the present self-administration experiments, AWD 131-138 was not persistently self-administered by monkeys above vehicle levels, even though the dose was increased up to 100 μg/kg per injection. At the 100-μg/kg injection dose of AWD 131-138, lever press responding did remain at cocaine levels during the first daily session and monkeys intravenously self-administered 24–39 injections during the session (a mean total intake of 2.4–3.9 mg/kg AWD 131-138 in 1 h). This was estimated to produce plasma levels of about 849–1041 ng/ml by the end of the first self-administration session, which corresponded to the plasma levels needed to produce anticonvulsive and anxiolytic activity in rats and anticonvulsive activity in dogs (Tober, unpublished observations). During the second and third sessions at the 100-μg/kg dose per injection of AWD 131-138, however, rates of responding and numbers of injections per session markedly decreased. Although a wide range of benzodiazepines, including midazolam and diazepam, has been shown to be self-administered by monkeys when substituted for cocaine (Bergman and Johanson, 1985; Griffiths et al., 1981, 1991), benzodiazepines appear to be less effective in maintaining self-administration behavior in cocaine-trained monkeys than in barbiturate-trained monkeys (Bergman and Johanson, 1985). Additional AWD 131-138 substitution studies under different access conditions and in animals trained to self-administer drugs other than cocaine, such as barbiturates or benzodiazepines (e.g., Munzar et al., 2001), are needed.

In conclusion, results of the drug discrimination study in squirrel monkeys indicate that AWD 131-138 does not produce benzodiazepine-like stimulus effects in humans. Also, AWD 131-138 failed to maintain i.v. self-administration behavior by monkeys when substituted for cocaine. However, the possibility that AWD 131-138 would be self-administered by monkeys with an extensive history of sedative self-administration cannot be fully excluded. Taken together, the results of the present experiment support previous neurochemical findings of limited activity of AWD 131-138 at benzodiazepine binding sites and suggest that it would not have benzodiazepine-like abuse liability in humans.

Acknowledgements

We thank Dr. Steven R. Goldberg for advice on the conduct of the experiments and for critical reading of the manuscript. This work was supported by U.S. PHS grants 03774, 10566, and 11453 and, in part, by Arzneimittelwerk Dresden and the Intramural Research Program of the National Institute on Drug Abuse, National Institutes of Health, Department of Health and Human Services.

References

Balster, R.L., 1991. Drug abuse potential evaluation in animals. Br. J. Addict. 86, 1549–1558.

Bergman, J., Johanson, C.E., 1985. The reinforcing properties of diazepam under several conditions in the rhesus monkey. Psychopharmacology 86, 108–113.

Bialer, M., Johannessen, S.I., Kupferberg, H.J., Levy, R.H., Loiseau, P., Perucca, E., 1999. Progress report on new antiepileptic drugs: a summary of the fourth EILAT conference (EILAT IV). Epilepsy Res. 34, 1–41.

Bialer, M., Johannessen, S.I., Kupferberg, H.J., Levy, R.H., Loiseau, P., Perucca, E., 2001. Progress report on new antiepileptic drugs: a summary of the fifth EILAT conference (EILAT IV). Epilepsy Res. 43, 11–58.

Colpaert, F.C., Desmedt, C.K.C., Jannsen, P.A.J., 1976. Discriminative stimulus properties of benzodiazepines, barbiturates, and pharmacologically related drugs: relation to some intrinsic anticonvulsant effects. Eur. J. Pharmacol. 37, 113–123.

Garcha, H.S., Rose, I.C., Stolerman, I.P., 1985. Midazolam cue in rats: generalization tests with anxiolytic and other drugs. Psychopharmacology 87, 233–237.

Goldberg, S.R., 1973. Comparable behavior maintained under fixed-ratio and second-order schedules of food presentation, cocaine injection or D-amphetamine injection in the squirrel monkey. J. Pharmacol. Exp. Ther. 186, 18–30.

Griffiths, R.R., Weerts, E.M., 1997. Benzodiazepine self-administration in humans and laboratory animals—implications for problems of long-term use and abuse. Psychopharmacology 134, 1–37.

Griffiths, R.R., Lukas, S.E., Braddford, L.D., Brady, J.V., Snell, J.D., 1981. Self-injection of barbiturates and benzodiazepines in baboons. Psychopharmacology 75, 101–109.

Griffiths, R.R., Lamb, R.J., Sannerud, C.A., Ator, N.A., Brady, J.V., 1991. Self-injection of barbiturates, benzodiazepines and other sedative–anxiolytics in baboons. Psychopharmacology 103, 154–161.

Heinecke, K., Thiel, W., 2001. Identity and physicochemical properties of 1-(4-chlorophenyl)-4-morpholino-imidazolin-2-one, AWD 131-138. Pharmazie 56, 458–461.

Jain, K.K., 2000. An assessment of rufinamide as an anti-epileptic in comparison with other drugs in clinical development. Expert Opin. Investig. Drugs 9, 829–840.

Johanson, C.E., Balster, R.L., 1978. A summary of results of a drug self-administration study using substitution procedures in rhesus monkeys. Bull. Narc. 30, 43–54.

Katz, J.L., Winger, G.D., Woods, J.H., 1991. Abuse liability of benzodiazepines and 5-HT_{1A} agonists. In: Rodgers, R.J., Cooper, S.J. (Eds.), 5-HT_{1A} Agonists, 5-HT_3 Antagonists and Benzodiazepines: Their Comparative Behavioural Pharmacology. Wiley, West Sussex, UK, pp. 317–341.

Munzar, P., Yasar, S., Redhi, G., Justinova, Z., Goldberg, S.R., 2001. High rates of midazolam self-administration in squirrel monkeys. Behav. Pharmacol. 12, 257–266.

National Research Council, 1996. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C.

Rostock, A., Tober, C., Dost, R., Bartsch, R., 1998a. Anxiolytic effects without sedation and amnesia of AWD 131-138 in animals: a comparison with diazepam and buspirone. Naunyn-Schmiedeberg's Arch. Pharmacol. 357 (Suppl. 4), R97.

Rostock, A., Tober, C., Dost, R., Rundfeldt, C., Bartsch, R., Egerland, U., Stark, B., Schupke, H., Kronbach, T., Lankau, H.-J., Unverferth, K., Engel, J., 1998b. AWD 131-138, ADD-233089. Anxiolytic, anticonvulsant. Drugs Future 23, 253–255.

Rundfeldt, C., Roeper, J., Netzer, R., 1999. AWD 131-138 antagonizes the corticotrophin releasing factor in locus coeruleus neurons in murine brain stem slices. Naunyn-Schmiedeberg's Arch. Pharmacol. 359 (Suppl. 3), R97.

Sanger, D.J., Benavides, J., 1993. Discriminative stimulus effects of *w* (BZ) receptor ligands: correlation with in vivo inhibition of [3H]-flumazenil binding in different regions of the rat central nervous system. Psychopharmacology 111, 315–332.

Sannerud, C.A., Ator, N.A., 1995. Drug discrimination analysis of midazolam under a three-lever procedure: II. Differential effects of benzodiazepine receptor antagonists. J. Pharmacol. Exp. Ther. 275, 183–193.

Sigel, E., Baur, R., Netzer, R., Rundfeldt, C., 1998. The antiepileptic drug AWD 131-138 stimulates different recombinant isoforms of the GABA-A receptor through the benzodiazepine binding site. Neurosci. Lett. 245, 85–88.

Spealman, R.D., 1985. Discriminative-stimulus effects of midazolam in squirrel monkeys: comparison with other drugs and antagonism by Ro 15-1788. J. Pharmacol. Exp. Ther. 235, 456–462.

Spear, D.J., Muntaner, C., Goldberg, S.R., Katz, J.L., 1991. Methohexital and cocaine self-administration under fixed-ratio and second-order schedules. Pharmacol. Biochem. Behav. 38, 411–416.

Tanda, G., Munzar, P., Goldberg, S.R., 2000. Self-administration behavior is maintained by the psychoactive ingredient of marijuana in squirrel monkeys. Nat. Neurosci. 3, 1073–1074.

Tidey, J.W., Bergman, J., 1998. Drug discrimination in methamphetamine-trained monkeys: agonist and antagonist effects of dopaminergic drugs. J. Pharmacol. Exp. Ther. 285, 1163–1174.

Tober, C., Rostock, A., Bartsch, R., 1998. Anticonvulsant profile of AWD 131-138 a derivative of a series of imidazolinones. Naunyn-Schmiedeberg's Arch. Pharmacol. 357 (Suppl. 4), R98.

Tober, C., Rostock, A., White, H.S., Wolf, H.H., Bartsch, R., 1999. Anticonvulsant activity of AWD 131-138 in genetic animal models of epilepsy. Naunyn-Schmiedeberg's Arch. Pharmacol. 359 (Suppl. 3), R97.

Tober, C., Stark, B., Bartsch, R., Kronbach, T., 2000. Effect of AWD 131-138 in a model for focal epilepsy, the amygdala kindling model in rats. Epilepsia 41 (Suppl. 7), 1.189.

Walker, M.C., Sander, J.W., 1998. New anti-epileptic drugs. Expert Opin. Investig. Drugs 8, 1497–1510.

Woolverton, W.L., Nader, M.A., 1990. Experimental evaluation of the reinforcing effects of drugs. Modern Methods in Pharmacology. Testing and Evaluation of Drugs of Abuse, vol. 6. Wiley-Liss, New York, pp. 165–192.

Woudenberg, F., Slangen, J.L., 1989. Discriminative stimulus properties of midazolam: comparison with other benzodiazepines. Psychopharmacology 97, 466–470.

Yasar, S., Bergman, J., 1994. Amphetamine-like effect of *l*-deprenyl (selegiline) in drug discrimination studies. Clin. Pharmacol. Ther. 56, 768–773.

Young, A.M., Herling, S., 1986. Drugs as reinforcers: studies in laboratory animals. In: Goldberg, S.R., Stolerman, I.P. (Eds.), Behavioral Analysis of Drug Dependence. Academic Press, Orlando, FL, pp. 9–67.