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Sarmazenil-Precipitated Withdrawal: A Reliable Method for Assessing Dependence Liability of Benzodiazepine Receptor Ligands

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MARTIN, J. R., J.-L. MOREAU, F. JENCK AND L. PIERI. *Sarmazenil-precipitated withdrawal: A reliable method for assessing dependence liability of benzodiazepine receptor ligands.* PHARMACOL BIOCHEM BEHAV **59**(4) 939–944, 1998.—The benzodiazepine receptor partial inverse agonist sarmazenil exhibits in vivo proconvulsive, but not convulsant, effects in different paradigms in rodents. Intravenous sarmazenil challenge given at several fixed intervals following the termination of repeated treatment with a markedly sedative dose of diazepam in squirrel monkeys was effective in precipitating withdrawal signs, but had no comparable effects in vehicle-treated controls. The precipitated withdrawal reaction was not only robust, but it was consistently observed in all of the diazepam-treated monkeys. Thus, the use of sarmazenil challenge in the precipitated withdrawal paradigm provides a reliable method for assessing the development of physical dependence during repeated treatment with benzodiazepine receptor agonists. © 1998 Elsevier Science Inc.

Diazepam dependence Precipitated withdrawal Sarmazenil Diazepam Benzodiazepine Tolerance

THE precipitated withdrawal paradigm involves repeated treatment with receptor agonists followed by challenge with a specific antagonist for that receptor to abruptly and completely block the agonistic activity and, thereby, elicit withdrawal signs, depending upon the magnitude of underlying physical dependence. With the discovery of the benzodiazepine receptor (BZR) antagonist flumazenil (6), the way was open to applying this method to the investigation of dependence produced by BZR full agonists (9). Although several other BZR antagonists have been used occasionally as pharmacological challenge in experiments with animals dependent on any of several BZR agonists (3,11), most investigations have relied upon flumazenil (17). In preliminary experiments in monkeys, challenge with the BZR partial inverse agonist sarmazenil (7-chloro analog of flumazenil) appeared to provide a more sensitive method for detecting underlying physical dependence because flumazenil challenge failed to elicit convulsions following repeated treatment with the BZR partial agonist bretazenil, whereas sarmazenil challenge was shown to be effective in precipitating convulsions (10,16). In vitro experimental results have characterized sarmazenil as a weak BZR inverse agonist; it inhibited GABA-induced chloride current in frog sensory neurons (18) and reduced GABA-induced chloride flux in a membrane preparation of rat cerebral cortex (15). In view of these results and the prominence of convulsions in the benzodiazepine withdrawal syndrome under the extreme experimental conditions used in animals, a first set of experiments investigated the convulsant/proconvulsant effects of sarmazenil in vivo in several rodent models. Intrinsic convulsant activity of sarmazenil would make the interpretation of benzodiazepine withdrawal reaction difficult. Second, having shown that sarmazenil given alone was not convulsant, this compound was then used to produce withdrawal in diazepam-treated squirrel monkeys to investigate both the consistency and magnitude of sarmazenil precipitated withdrawal.

METHOD

Animals and Maintenance Conditions

Female albino mice (Ibm: MORO) and female albino rats (Ibm: RORO) were used in the pentylenetetrazol tests. Male albino rats were used in the isoniazid experiments (Ibm: RORO). Young female mice (Ibm: DBA/2J) were used in the experiments on audiogenic seizures. All rodents were obtained from Biological Research Laboratories (CH-4414 Füllinsdorf, Switzerland). The mice and rats were delivered to the laboratory colony at least 1 day prior to testing. These animals were housed in group cages with sawdust bedding under standard maintenance conditions (12 L:12 D cycle; 21–23°C; 55–65% relative humidity). Both mice and rats received standard laboratory chow and tap water ad lib in the home cage.

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Testing was done between 0700 and 1800 h. At the conclusion of testing the rodents were euthanized by CO_2 exposure.

Adult male squirrel monkeys (*Saimiri sciureus)* were used. All animals were drug experienced and had been in the laboratory colony for several years. Prior to the start of the present experiment the monkeys had received no acute drug treatment for at least 1 month, and no repeated treatment with experimental compounds for at least 3 months. Throughout the treatment regimen, the monkeys were individually housed in large stainless steel cages equipped with grid floor and two elevated perches and located in a colony room with visual contact to conspecifics. Both the temperature (28–30°C) and the humidity (50–60%) of the animal quarters were regulated. A 12 L:12 D cycle with light onset at 0600 h was used. A dry, nutritionally sufficient diet was available ad lib and was supplemented daily with fresh fruits and vegetables. A water bottle was attached to each cage to provide continuous access. Testing was performed between 0700 and 1800 h.

Experimental Procedures

Convulsion tests in rodents. Potentiation of the effect of a threshold convulsant dose of pentylenetetrazol (PTZ; 60 mg/kg, IP) was evaluated in mice and rats. Sarmazenil or vehicle was administered 15 min prior to PTZ injection and the animals observed in the home cage to provide information on the intrinsic effects of sarmazenil. Following the PTZ injection each animal was observed for a further 30 min in an open glass cylinder containing only sawdust bedding (providing results on potential proconvulsive effects of sarmazenil). Typically, only about 20% of vehicle-treated animals challenged with this dose of PTZ exhibited tonic convulsions. Each animal was euthanized with CO_2 immediately after exhibiting tonic convulsions or, in the absence of any tonic convulsions, at the end of the observation period. Groups of 80 mice (PO administration) and 12 rats (IV administration) were used to evaluate PTZ combined with the vehicle condition, as well as in combination with each of several doses of sarmazenil. The proportion of animals in each treatment group exhibiting tonic convulsions was determined. In a further experiment, vehicle or sarmazenil (1 and 10 mg/kg) was given IV alone in rats and the animals observed for 30 min for tonic convulsions.

Potentiation by sarmazenil of isoniazid-induced clonic–tonic convulsions was evaluated in rats. Sarmazenil or its vehicle was administered either PO or IV 15 min after injection of isoniazid (320 mg/kg, SC). This represented a just supraliminal dose of of the convulsant isoniazid, which, given alone, elicits clonic–tonic convulsions in approximately 10% of the rats; such convulsions were previously reported to occur with a latency of at least 30 min (14). The rats were observed in an open glass cylinder with sawdust bedding for 2 h after isoniazid injection and the occurrence of clonic–tonic convulsions noted. The proportion of each treatment group exhibiting convulsions was calculated. Groups of 20 rats per dosage condition were used (with parallel groups of vehicle-treated animals). Each animal was euthanized with CO_2 immediately after exhibiting convulsions or, in the absence of convulsions, at the end of the observation period.

Potentiation of audiogenic seizures elicited by subconvulsant intensity of acoustic stimulation was investigated in 21-day-old DBA/2J mice (which are known to be maximally seizure susceptible). Intraperitoneal treatment with sarmazenil or vehicle was given 30 min prior to testing. Immediately following treatment, each mouse was placed in a separate open Plexiglas box (21 × 44 × 21 cm) containing sawdust bedding and observed closely during the 30-min pretest interval. Testing was then carried out in a sound-isolated chamber. The mouse was exposed to a 14 kHz sinusoidal tone at 90 dB (zero dB was defined as a pressure level of 20 mPa) for 1 min, during which observations were made. Following vehicle treatment, such acoustic stimulation typically induced wild running, clonic seizures, and/or tonic convulsions in only about 10% of the mice tested. Groups of 10 mice were used to evaluate the effect of vehicle and each of several doses of sarmazenil (0.1–10 mg/kg). The proportion of each group exhibiting tonic convulsions was recorded. Each mouse was euthanized with CO_2 immediately after exhibiting tonic convulsions or, in the absence of convulsions, at the termination of acoustic stimulation.

Behavioral observation in monkeys. Following a single IV injection of sarmazenil (3 and 5 mg/kg), each monkey was returned to its individual home cage and observed periodically over the subsequent 2 h. Behavior observations were recorded by a trained observer using a standard classification system, but emphasis was placed on the detection of convulsions. Each dose was tested in three monkeys.

Sarmazenil challenge following repeated administration of vehicle or diazepam. Different groups of monkeys received oral treatment once daily for 11 consecutive days with either 30 mg/kg diazepam (n = 18) or vehicle (n = 9). Treatment was given at the same time each morning and the behavior of each monkey was observed closely in the home cage during the 1-h period postadministration to permit the assessment of tolerance development to two diazepam-induced effects: loss of righting reflex and sleep posture.

Evaluation of physical dependence was done as follows. At several preselected time intervals (5, 24, and 48 h) after the final treatment administration with either vehicle or diazepam, each monkey was injected IV with 0.25 mg/kg sarmazenil (infused as a bolus over a period of about 10–15 s). The monkeys were observed in their home cage for withdrawal signs for a 2-h period after each of the three consecutive challenges. The observer was blind to the experimental conditions. In a few instances in which maximal withdrawal reactions occurred after an initial sarmazenil challenge, subsequent challenge tests were not carried out to minimize possible deleterious effects to the health of the animal. The results for each individual monkey during the 12 10-min blocks within each 2-h challenge test were assigned a severity score for tremors, vomiting, and convulsions separately. Weighed scoring was done as follows: tremor duration (1–4 blocks = 0; 5–8 blocks = 1; 9–12 blocks = 2) plus tremor intensity (weak = 1; moderate = 2; pronounced = 3); vomiting duration (1–4 blocks = 1; 5–8 blocks = 2; 9–12 blocks = 3); convulsion duration (1–2 blocks = 1; 3–4 blocks = 2; 5–6 blocks = 3; 7–8 blocks = 4; 9–10 blocks = 5; 11–12 blocks = 6) plus convulsion intensity (weak = 2; moderate = 4, pronounced = 6). The scores for duration and intensity (only available for tremors and convulsions) were added to yield a total score for each of the three responses assessed, with emphasis placed on determining for each individual monkey the single challenge test in which the maximal withdrawal reaction occurred based on the combination of scores for the three withdrawal signs. This maximal precipitated withdrawal reaction for each individual monkey was then used as the basis for subsequent analysis.

Statistical Evaluation

Statistical significance of comparisons between vehicle-treated and sarmazenil-treated rodents in each of the convul-

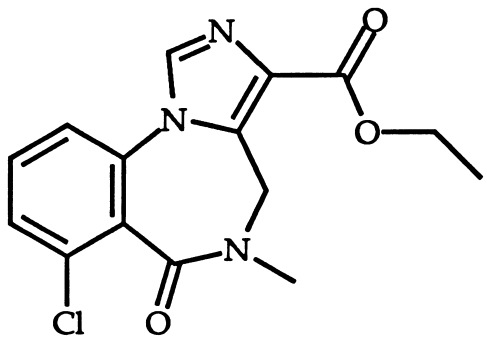

FIG. 1. Chemical structure of sarmazenil.

sion tests (PTZ, isoniazid, audiogenic seizures) was evaluated with a one-tailed chi-square test. In monkeys, tolerance was assessed by comparison of the latency to occurrence of loss of righting reflex or sleep posture on the first day vs. the final day of diazepam treatment using a one-tailed Wilcoxon matched-pairs, signed-ranks test. Comparison of the different precipitated withdrawal signs in diazepam-treated vs. vehicle-treated monkeys was done with a one-tailed Mann–Whitney *U*-test. *A p*-value $\leqslant 0.05$ was accepted as statistically significant.

Drugs and Administration

The experimental drugs used were diazepam and sarmazenil (INN; company code Ro 15-3505; ethyl 7-chloro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5a] [1,4]benzo-diazepine-3-carboxylate; see Fig. 1). Both of these compounds were synthesized at F. Hoffmann–LaRoche Ltd. Sarmazenil was diluted as necessary with distilled water for administration in mice (10 ml/kg IP or PO), rats (2 ml/kg IV; 10 ml/kg PO), and monkeys (1 ml/kg IV). Diazepam (micronized powder) was suspended in a vehicle of 0.3% (v/v) Tween 80 in distilled water and administered orally to monkeys in a volume of 1 ml/kg body weight. Isoniazid and pentylenetetrazol were purchased commercially; both were dissolved in physiological saline and administered parenterally in 10 ml/kg. The compounds were prepared for injection immediately prior to use.

RESULTS

Proconvulsant Effects in Rodents

Neither tonic convulsions nor obvious signs of altered spontaneous behavior were observed during the 15-min period immediately following administration of sarmazenil (which preceded PTZ injection) for any of the doses of sarmazenil tested in mice (0.1, 1, and 10 mg/kg PO). However, in combination with PTZ challenge, the proportion of mice exhibiting tonic convulsions at the oral sarmazenil doses was significantly increased compared to vehicle treatment at the doses 0.1, 1, and 10 mg/kg PO, with a maximum of 69% of the mice exhibiting convulsions. These results are provided in Table 1.

No convulsions or other obvious behavioral alterations were observed in rats during a 30-min observation period following injection of sarmazenil at either 1 or 10 mg/kg IV. In addition, tonic convulsions were not observed during the 15-min period immediately following injection of sarmazenil (which preceded PTZ injection) for any of the doses of sarmazenil tested in rats (0.03, 0.1, 0.3, 1, 3, and 10 mg/kg IV). In rats given a threshold convulsant dose of PTZ, sarmazenil significantly increased the proportion of animals exhibiting tonic convulsions at the doses 1 to 10 mg/kg IV compared to vehicle, with a maximum of 75% of the rats exhibiting tonic convulsions. Tonic convulsions were not observed at any time in any of the rats treated with IV vehicle followed 15 min later by IP vehicle (instead of PTZ) and then further observed for 30 min. These results are provided in Table 1.

Isoniazid was first injected followed 15 min later by sarmazenil. Only 5% of the rats receiving vehicle IV or 20% receiving vehicle PO after isoniazid (given at the selected supraliminal dose) exhibited clonic–tonic convulsions. Sarmazenil produced a dose-dependent proconvulsant effect; the doses 0.03 to 0.3 mg/kg IV and 3 to 10 mg/kg PO produced a significant increase in the proportion of animals exhibiting convulsions. These results are provided in Table 1.

Sarmazenil failed to elicit convulsions or produce any other obvious behavioral alterations during the initial 30-min postinjection at any of the doses tested (0.01, 0.03, 0.1, 0.3, 1, 3, and 10 mg/kg IP) in the audiogenic seizure paradigm in young DBA/2J mice. In combination with acoustic stimulation there was a dose-related increase in the proportion of mice exhibiting tonic convulsions with maximally 60–80% of

TABLE 1

PROCONVULSANT EFFECT OF SARMAZENIL IN COMBINATION WITH PENTYLENETETRAZOL (PTZ) OR ISONIAZID IN RODENTS

	Pentylenetetrazol (PTZ) Test		Isoniazid Test	
Sarmazenil dose (mg/kg)	Mice (PO Administration): Percentage of the Treated Animals Exhibiting Tonic Convulsions After PTZ	Rats (IV Administration): Percentage of the Treated Animals Exhibiting Tonic Convulsions After PTZ	Rats (PO Administration): Percentage of the Treated Animals Exhibiting Tonic Convulsions After Isoniazid	Rats (IV Administration): Percentage of the Treated Animals Exhibiting Tonic Convulsions After Isoniazid
Vehicle	14% ($n = 80$)	25% ($n = 12$)	20% ($n = 20$)	5% ($n = 20$)
0.01	ND	ND	ND	20% ($n = 20$)
0.03	ND	25% ($n = 12$)	ND	65% ($n = 20$)†
0.1	30% ($n = 80$)*	25% ($n = 12$)	ND	80% ($n = 20$)†
0.3	ND	50% ($n = 12$)	35% ($n = 20$)	85% ($n = 20$)†
1.0	69% ($n = 80$)†	75% ($n = 12$)*	ND	ND
3.0	ND	75% ($n = 12$)*	95% ($n = 20$)†	ND
10	69% ($n = 80$)†	75% ($n = 12$)*	90% ($n = 20$)†	ND

ND = not determined. The number of animals tested under each treatment condition is shown within parentheses. Statistical significance of comparisons between vehicle-treated and sarmazenil-treated animals was evaluated with the one-tailed chi-square test (*$p \leqslant 0.05$, †$p \leqslant 0.01$).

the mice exhibiting tonic convulsions in the dose range 1–10 mg/kg. In comparison, only 10% of vehicle-treated mice exhibited tonic convulsions during acoustic stimulation. The doses 1 and 10 mg/kg IP produced a significant increase in the proportion of animals exhibiting convulsions ($ps \leq 0.01$). These results are illustrated in Fig. 2.

Effects on Spontaneous Behavior in Monkeys

Sarmazenil was intravenously injected at the doses 3 mg/kg and 5 mg/kg in squirrel monkeys to determine its effects on spontaneous behavior. At the dose of 3 mg/kg, two out of the three animals tested refused a preferred food (which was normally avidly grabbed and consumed) and appeared less active than usual, whereas the third animal showed no clear behavioral alterations. At the dose 5 mg/kg, all three animals that were evaluated refused a preferred food and were less active than normal; one of these monkeys retched and vomited during the first several minutes and again about 1 h postinjection. The effects observed at these two selected IV doses of sarmazenil were most clearly observed during about the initial half hour after injection. No signs indicative of possible convulsions were observed.

Tolerance Development in Diazepam-Treated Monkeys

Tolerance develops to various CNS depressant effects of BZR agonists, for example, the loss of righting reflex and sleep (5,9). The dose of 30 mg/kg diazepam administered orally produced loss of righting reflex in all but one of 18 monkeys on the first treatment day, in only 6 of the monkeys on the second treatment day, in only 2 of the monkeys on the third treatment day, and thereafter no loss of righting reflex was observed in any of the monkeys. The estimated magnitude of this response was maximal in most monkeys after the first diazepam treatment (13 out of 18), with weaker responses observed subsequently. In comparison, the development of tolerance to the sleep-inducing effect of diazepam was less rapid with all 18 monkeys exhibiting a sleep posture after the initial diazepam treatment and successively ever fewer monkeys on the following treatment days, with less than half of the monkeys exhibiting sleep posture during the observation period after treatment day 5, and only one monkey doing so on the final treatment day. About half of these monkeys exhibited sleep posture after the initial diazepam treatment, but subsequently none of the monkeys did so. There was a significant increase in the latency to the first occurrence of loss of righting reflex ($T = 0, p \leq 0.01$) and sleep posture ($T = 0, p \leq 0.01$) from the first day to the final day of diazepam treatment. Figure 3 shows the mean latency (±SEM) to the occurrence of loss of righting reflex and sleep posture following each daily diazepam treatment. During the 11-day treatment regimen, oral vehicle treatment failed to induce loss of righting reflex or sleep posture during the 1-h observation period postinjection.

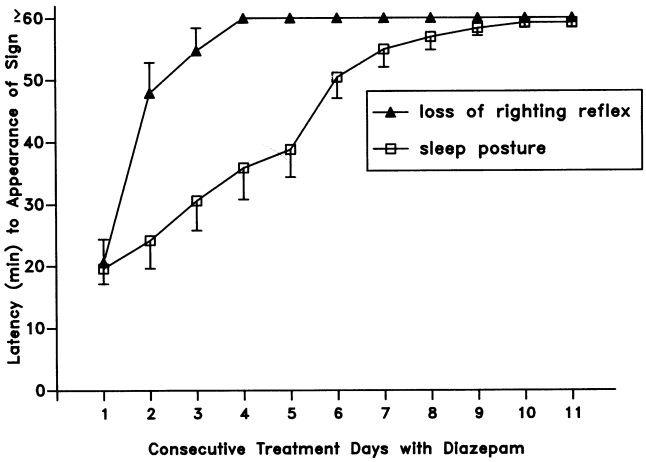

FIG. 3. Tolerance development for diazepam-induced loss of righting reflex (solid triangles) and sleep posture (empty squares) in monkeys ($n = 18$). Values are shown as mean ± SEM. Evaluation with a one-tailed Wilcoxon test showed that the latency to the occurrence of loss of righting reflex and sleep posture significantly increased from the first day to the final day of treatment ($ps \leq 0.01$).

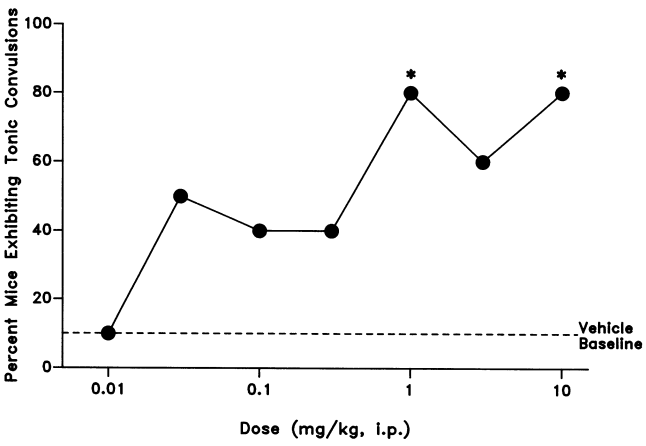

FIG. 2. Proconvulsant effect of sarmazenil in the audiogenic seizure test in DBA/2J mice. The percentage of mice (out of groups of 10) exhibiting tonic convulsions is shown. Statistical significance of comparisons between vehicle-treated and sarmazenil-treated groups was evaluated with a one-tailed chi-square test. $*p \leq 0.01$.

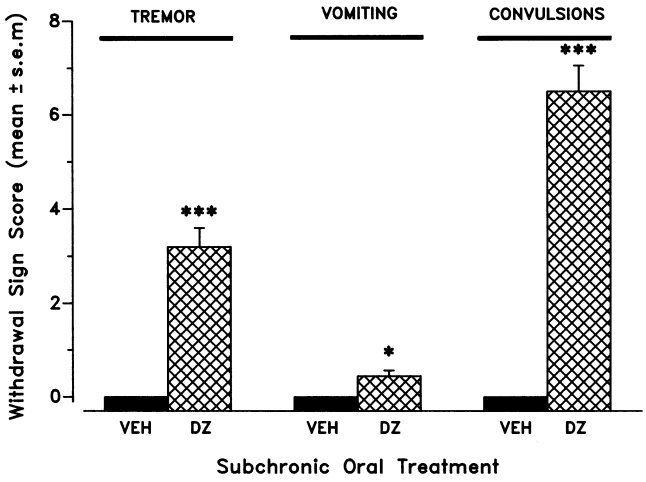

FIG. 4. Sarmazenil challenge in monkeys subchronically treated with vehicle (solid bars) or diazepam (crosshatched bars). The scores (mean ± SEM) for the precipitated withdrawal signs tremor, vomiting, and convulsions are shown. Evaluation with a one-tailed Mann–Whitney *U*-test showed that all three signs were significantly more marked in diazepam-treated ($n = 18$) vs. vehicle-treated monkeys ($n = 9$). $*p \leq 0.05$; $***p \leq 0.001$.

Precipitated Withdrawal in Diazepam-Treated Monkeys

Several typical signs of precipitated withdrawal have been identified in squirrel monkeys treated subchronically with different BZR agonists; these include tremor, vomiting, and convulsions (9). In the present experiment, 0.25 mg/kg sarmazenil was injected IV at each of three time points (5, 24, and 48 h) following the final oral diazepam or vehicle treatment of the subchronic regimen. The occurrence of sarmazenil-induced tremor, vomiting, and convulsions during the subsequent 2-h period was noted (latency and an estimate of the response magnitude). Seventeen out of the 18 diazepam-treated monkeys challenged with sarmazenil exhibited convulsions in at least one of the three challenge tests; in contrast, none of the nine vehicle-treated monkeys did so in any of the challenge tests. The maximal precipitated withdrawal reaction (based on the previously described scoring system applied to individual animals) observed in diazepam-treated monkeys was observed in four monkeys after the first challenge, in eight monkeys after the second challenge, and in six monkeys after the third challenge with sarmazenil. The maximal magnitude of the precipitated convulsions observed in the diazepam-treated monkeys in any of these challenge tests was found to be pronounced in 12 monkeys, moderate in 3 monkeys, and weak in 2 monkeys (only a single monkey failed to exhibit any convulsions). Typically such convulsions were only of several minute's duration, occurring sporadically during a period of about 10–30 min. Vomiting occurred in 15 out of the 18 diazepam-treated monkeys in at least one challenge test, but in none of the vehicle-treated monkeys challenged with sarmazenil. Tremor was also elicited by sarmazenil in all 18 diazepam-treated monkeys, and was typically observed in all of the challenge tests for an individual monkey. In contrast, tremor was not observed during challenge tests in vehicle-treated monkeys. The maximal magnitude of the sarmazenil-precipitated tremor in diazepam-treated monkeys occurring in any of the three challenge tests was found to be pronounced in 13 monkeys, moderate in 4 monkeys, and weak in 1 monkey.

Figure 4 illustrates the mean scores (±SEM) for sarmazenil-induced vomiting, tremor, and convulsions obtained in both the diazepam-treated and the vehicle-treated groups (for the single challenge test in each monkey in which the maximal total score was obtained). It is unlikely that this represented spontaneous withdrawal because the median latency to the occurrence of the first convulsion following sarmazenil challenge was only 3 min (maximum latency 12 min for the 17 monkeys that exhibited convulsions). The withdrawal signs observed after sarmazenil challenge were significantly more pronounced in the diazepam-treated group than in the vehicle-treated group: tremor ($U = 4.5$, $p \leq 0.001$), vomiting ($U = 45$, $p \leq 0.05$), convulsions ($U = 4.5$, $p \leq 0.001$). During the 2 weeks following the challenge tests, the monkeys were observed at least once daily for signs of spontaneous withdrawal but none were noted.

DISCUSSION

Sarmazenil (7-chloro analog of flumazenil) exhibits specific high-affinity binding to the BZR ($IC_{50} = 2.7$ nM for displacement of [^{3}H]diazepam in rat cerebral cortical membranes; unpublished data). It has been characterized in vitro as a weak BZR inverse agonist, inhibiting GABA-induced chloride current in frog sensory neurons (18) and reducing GABA-induced chloride flux in rat cerebral cortical membrances (15). Sarmazenil reversed the sedative effects of BZR agonists in various species (4,10). The limited proconvulsant effect of sarmazenil obtained here in three different experimental paradigms in mice and rats, with some animals failing to exhibit convulsions even at very high doses, is consistent with its described partial inverse agonism. Sarmazenil was not convulsant in rodents because it failed to elicit convulsions when given alone. When injected IV in squirrel monkeys at up to 20 times the dose used in the challenge tests done here, sarmazenil produced only minor and inconsistent behavioral effects and, in particular, no evidence whatsoever of convulsions. In healthy volunteers, IV sarmazenil reversed the amnestic effects of flunitrazepam (2) and the electroencephalographic effects of midazolam (1). In sleep-deprived volunteers, sarmazenil exhibited an activating effect (12).

In a previous study in squirrel monkeys it was demonstrated that flumazenil (10 mg/kg, IV) was effective in precipitating withdrawal signs in monkeys treated with several different BZR full agonists (9). However, only a portion of the benzodiazepine-dependent monkeys (including diazepam-treated monkeys) exhibited flumazenil-induced convulsions (which provide a clear sign of the underlying dependence). Furthermore, even higher doses of flumazenil failed to increase the proportion of diazepam-dependent monkeys exhibiting precipitated convulsions in this paradigm (unpublished results). In a comparison of several different BZR partial inverse agonists and antagonists (including flumazenil) administered to small groups of monkeys at equieffective antagonistic doses, sarmazenil was shown to be the most effective in precipitating diazepam withdrawal (8). The present experiment confirmed this finding in a large group of diazepam-treated monkeys; sarmazenil precipitated convulsions in 17 out of the 18 diazepam-treated monkeys, but in none of the nine vehicle-treated monkeys. The high sensitivity of this dependence paradigm using sarmazenil to elicit withdrawal signs is supported by the previous demonstration that sarmazenil, but not BZR antagonist flumazenil, was effective in precipitating convulsions in squirrel monkeys subchronically treated with high doses of the BZR partial agonist bretazenil (10,16).

It has been reported that chronic treatment with BZR agonists in animals produces a so-called "withdrawal shift" at the receptor level resulting in attenuation of BZR agonist actions and enhancement of BZR inverse agonist actions (13). The BZR antagonist flumazenil, in fact, became proconvulsant after chronic flurazepam treatment (7). In the present experiment, sarmazenil may have become even more proconvulsant due to development of physical dependence to chronically administered diazepam. This may be responsible for the robust precipitated withdrawal reaction observed, which included marked seizures. Taken together with the published literature, the present results indicate that use of the BZR partial inverse agonist sarmazenil to precipitate a withdrawal reaction in animals treated repeatedly with BZR agonists or partial agonists appears to provide an effective and reliable method for the evaluation of physical dependence liability.

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