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BRIEF COMMUNICATION

A Simple and Rapid Method of Inducing Physical Dependence With Benzodiazepines in Mice

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PATEL, J. B., C. A. RINARELLI AND J. B. MALICK. *A simple and rapid method of inducing physical dependence with benzodiazepines in mice.* PHARMACOL BIOCHEM BEHAV **29**(4) 753–754, 1988.—Physical dependence was rapidly induced in mice by administering diazepam intraperitoneally twice daily using an incremental dosing regimen (50 to 450 mg/kg) for nine consecutive days. Withdrawal was induced (24 hr after the last dose) by administration of a benzodiazepine antagonist, RO-15-1788 (10 mg/kg, IP). All of the mice exhibited clear-cut withdrawal symptoms (i.e., convulsions) within minutes of antagonist treatment. This method offers a simple, reliable, high throughput procedure for the assessment of benzodiazepine-like physical dependence liability and withdrawal, and it would be useful for screening purposes.

Diazepam Precipitated withdrawal RO-15-1788 Mice Physical dependence Anxiolytics

PHYSICAL dependence on benzodiazepines (BZ) has been difficult to demonstrate unless these agents were administered chronically for long periods of time; typically, these drugs must be given continuously (e.g., in the diet [2], drinking water [1], or gastric fistula [4]) for periods of at least four weeks or have used species such as rhesus monkeys [6] and baboons [3] which require highly specialized and expensive facilities. In addition, for the most part, the withdrawal phenomena observed have been mild and difficult to score because of their subjective nature (e.g., tremors) or the methods have used convulsants to precipitate hyperexcitability [7]. The present report describes a simple procedure for inducing BZ physical dependence in mice and for precipitating clear-cut, readily observable withdrawal symptoms (i.e., convulsions).

METHOD

Male Swiss-Webster mice (Hilltop Laboratories, PA, weighing 18–20 g at initiation of study) were used in these studies. Five mice were housed per cage, and they received food and water ad lib. Drugs were suspended in a HPMC vehicle (0.1% Tween 80, 0.5% hydroxymethylcellulose in 0.9% NaCl), and the volume of each injection was 10 ml/kg of body weight.

Subjects were treated intraperitoneally with either vehicle or diazepam twice daily (8 a.m. and 4 p.m.) for nine consecutive days. Initially, on day 1, diazepam-treated subjects received a dose of 50.0 mg/kg twice daily, and the dose of diazepam was increased daily by 50.0 mg/kg for nine consecutive days (450 mg/kg twice daily on day 9). On day 10, precipitated withdrawal was induced (24 hr after the last dose) by administration of RO-15-1788 (10 mg/kg, IP), and the mice were observed for the next 30 min. It should be mentioned that 10 percent of the mice died during the drug treatment period. At least five mice were used for each withdrawal study or gross observation.

RESULTS

The results obtained when precipitated withdrawal was induced by RO-15-1788 (10.0 mg/kg, IP) in mice treated with incremental doses of diazepam for nine consecutive days are presented in Table 1. Clonic convulsions were noted in 100% of the mice within five min. When a lower dose of RO-15-1788 (2.5 mg/kg, IP) was used, it caused convulsions in 60% of the mice tested (Table 1). In contrast, when a relatively low dose of diazepam (5.0 mg/kg, PO) or vehicle was administered instead of the RO-15-1788, there was no incidence of convulsions. It should be noted that when subjects were treated with incremental doses of diazepam for only four days and then treated with RO-15-1788 (10.0 mg/kg, IP) 24 hr later, the subjects all failed to show any withdrawal symptoms (Table 1).

In a separate series of experiments, the withdrawal interval (i.e., time between the last dose of diazepam and administration of RO-15-1788) was varied, and it was found that the 24 hr time point was the optimum time for onset of precipi-

TABLE 1

PRECIPITATED WITHDRAWAL INDUCED BY RO-15-1788 IN DIAZEPAM-TREATED MICE

Day	Diazepam (mg/kg, IP)[a]	Withdrawal Interval[b]	Antagonist Challenge (Dose, mg/kg, IP)	% Exhibiting Convulsions[c]
4	200	24 hr	RO-15-1788 (10.0)	0
9	450			
10	—	0 hr	Vehicle	0
		0 hr	RO-15-1788 (10.0)	0
		17 hr	RO-15-1788 (10.0)	66
		24 hr	Vehicle	0
		24 hr	Diazepam (5.0)	0
		24 hr	Metrazole (41.9)	0
		24 hr	RO-15-1788	
			(2.5)	60
			(5.0)	66
			(10.0)	100
		40 hr	RO-15-1788 (10.0)	60

[a]All mice were treated with the appropriate dose twice daily (8 a.m., 4 p.m.), starting at 50.0 mg/kg on day 1.

[b]Time between last dose of diazepam and treatment with RO-15-1788. Mice were challenged only once.

[c]All mice (N=5) were observed for 30 min following RO-15-1788 administration.

tated withdrawal (see Table 1). Table 1 also shows that chronic diazepam-treated mice, when challenged with vehicle or sub-threshold dose of metrazole (41 mg/kg, SC), failed to exhibit clonic convulsions; thus it did not appear that the metrazole seizure threshold was markedly altered.

DISCUSSION

The procedure described in the present report demonstrates that RO-15-1788 can precipitate a clear-cut withdrawal syndrome primarily characterized by convulsions (however, occasionally Straub tail was observed) in mice that have received diazepam for as short a period as nine consecutive days.

Several authors [1–4, 7] have studied benzodiazepine-induced physical dependence and precipitated withdrawal in animals; however, such studies, for the most part, exhibit one or more of the following major limitations: (1) they usually required long-term (>28 days) administration; (2) many incorporated benzodiazepines in the diet that required tedious mechanics (e.g., mixing drug and weighing food to approximate drug dosage intake; in addition, it is difficult to judge compound acceptability in food (e.g., taste aversion) by the subjects); (3) they require subjective evaluation of withdrawal symptoms (e.g., wet dog shakes, tremor, increased muscle tone, poker tail) that are difficult to quantify and the optimal time at which to observe their occurrence may not be consistent or known; (4) the use of relatively expensive species (e.g., baboons and rhesus monkeys) for which highly specialized research facilities are required; and (5) finally, the techniques may require minor surgery for the implantation of infusion mini-pumps or subcutaneous pellets. In addition, such procedures possess other technical problems in that unknown compounds have to be soluble in the infusion vehicle, which may limit the maximal dose that can be given.

In the present studies, it could be argued that the subjects' convulsive threshold was reduced following subacute treatment with diazepam. However, RO-15-1788 itself appears to exhibit some anticonvulsant activity under certain conditions [8], and the convulsant threshold did not appear to be altered when subconvulsive doses of metrazole were administered to separate groups of mice. Clinically, when withdrawal symptoms are observed on cessation of prolonged continuous administration of benzodiazepines, presumably the hyperexcitability reactions (e.g., tremors, agitation) that are observed are due to some as yet unknown alteration(s) in receptor sensitivity (e.g., at GABA sites) brought about as a result of adaptive changes in response to prolonged receptor occupancy. The precipitated withdrawal method has been widely used to determine physical dependence on opiate-like compounds [5], and the present method appears to be a useful method for performing similar evaluations with benzodiazepines.

Therefore, this procedure appears to offer a simple and rapid test for the detection of BZ-like physical dependence liability in mice that could be used as a primary screen.

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