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Lack of Tolerance Development to Benzodiazepines in Antagonism of the Pentylenetetrazol Discriminative Stimulus

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SHEARMAN, G. T., S. MIKSIC AND H. LAL. *Lack of tolerance development to benzodiazepines in antagonism of the pentylenetetrazol discriminative stimulus.* PHARMAC. BIOCHEM. BEHAV. **10**(5) 795–797, 1979.—In an operant procedure of lever pressing on FR 10 schedule of food reinforcement male hooded rats were trained to respond on a lever on one side of a food cup following a 20 mg/kg pentylenetetrazol (PTZ) injection and to respond on a lever on the alternate side following a 1 ml/kg saline injection. Upon acquisition of the PTZ-saline discrimination, diazepam and chlordiazepoxide were tested and found to antagonize the PTZ discriminative stimulus. The animals were then injected with 10 mg/kg diazepam or chlordiazepoxide for ten consecutive days. New dose-response curves obtained following this treatment indicated that tolerance did not develop to the antagonism of the PTZ discriminative stimulus by these benzodiazepines.

Drug discrimination Pentylenetetrazol Benzodiazepines Diazepam Chlordiazepoxide Tolerance

BENZODIAZEPINES are known for their antianxiety and depressant actions. Since tolerance develops only to the depressant action [3, 6, 13, 18, 20], lack of tolerance development to other actions in experimental animals has often been employed as a tool to identify pharmacological actions for their predictive value for clinical efficacy [4,12]. Recently, we have found that chlordiazepoxide and diazepam antagonize the discriminative stimulus property of subconvulsive dose of pentylenetetrazol (PTZ) [14–17]. In order to determine if the antagonistic property of these benzodiazepines is related to their psychotherapeutic action, we investigated whether tolerance would not develop to their action in the PTZ discrimination test. Previously lack of tolerance development to the prevention of PTZ-induced convulsions by diazepam has been reported [5, 8, 11].

METHOD

Animals

Male hooded rats of the Long-Evans strain (Charles River Breeding Laboratories, Wilmington, Mass.) weighing between 250–300 g at the beginning of the investigation were used. Animals were housed in single cages in a large colony room thermostatically maintained at 21 ± 1°C. Room lights were turned off from 8:00 p.m. to 8:00 a.m. Water was continuously available at the home cages but food was restricted to 20 g a day made available approximately 4 hr following each operant session.

Apparatus

The behavioral apparatus consisted of conventional Skinner boxes housed in lightproof, sound-attenuated and fan-ventilated chambers. Each Skinner box contained two levers, one on either side of a food cup and equidistant from the center. Scheduling of contingencies and recording of data was made by a combination of electromechanical and solid-state programming equipment.

Drug Administration

All injections were given IP in a constant volume of 1 ml/kg. Diazepam was homogenized in physiological saline containing 13% propylene glycol and 1% Tween 80. Chlordiazepoxide and pentylenetetrazol were dissolved in physiological saline.

Discrimination Training

The rats were first magazine trained and shaped to lever press for food reinforcement (for detailed procedure see Lal *et al.* [10]). The animals were then trained to press one of the levers 15 min following a 20 mg/kg PTZ injection and the other lever 15 min following a 1 ml/kg saline injection. Every tenth press (FR 10) on the appropriate lever resulted in the delivery of a 45 mg Noyes food pellet. Responses on the incorrect lever were recorded but did not result in the delivery of food. A possible effect of lever preference was counterbalanced by assigning the lever on the right side of the food cup to be the drug lever for half of the rats and the lever on the left side to be the drug lever for the remainder of the animals. To avoid the possibility that olfactory cues associated with the correct lever for rats previously tested in the chambers could serve as a cue the sequence of PTZ-saline injections was irregularly alternated for each group of

TABLE 1

EFFECT OF CHRONIC TREATMENT WITH BENZODIAZEPINES ON THE ANTAGONISM OF THE DISCRIMINATIVE STIMULUS PROPERTY OF PTZ

Drug*	Dose (mg/kg)	N	% Animals Selecting PTZ Lever† Acute	Chronic
Solvent	—	6	100	100
Diazepam	2.5	6	67	67
	10.0	6	17	33
Chlordiazepoxide	2.5	10	30	40
	10.0	10	20	20

*All animals were injected with PTZ (20 mg/kg) following drug administration and prior to placement in the test cages.

†Based upon responses prior to reinforcement.

rats. Training sessions were carried out 7 days a week according to an irregularly alternating sequence of injections. For each session, responses emitted on each lever prior to the first reinforcement were recorded as were the total responses emitted on each lever during the entire 10 min session.

When the animals attained a stable response rate and made not more than four responses on the incorrect lever (i.e., saline lever following PTZ injection) prior to the first reinforcement (10 responses on the correct lever) on nine out of ten consecutive sessions, antagonism testing was begun.

Antagonism Testing

For the acute study, these tests consisted of 10 min sessions separated by at least five practice sessions in which saline and PTZ were correctly discriminated. Test sessions were always followed by a practice session in which saline was injected. If the rats' performance on these practice sessions seemed to deteriorate (i.e. greater than 4 responses on the incorrect lever prior to the first reinforcement) further training sessions were given before testing was reinstated. The animals were injected with the appropriate dose of either diazepam, chlordiazepoxide or solvent to be followed 30 min later by PTZ (20 mg/kg). Fifteen minutes after the PTZ injection the animals were placed in their assigned Skinner boxes and allowed to respond on the lever of their choice until ten nonreinforced responses were completed on one of the levers. The lever on which ten responses were completed first was considered the selected lever. Following the acute study, several practice sessions were given in order to insure the maintainence of the PTZ-saline discrimination.

For the chronic study the animals that were tested acutely with diazepam were treated with diazepam (10 mg/kg) for ten consecutive days. Similarly, the animals tested acutely with chlordiazepoxide were treated with chlordiazepoxide (10 mg/kg). The remaining animals were treated with solvent. Results of other studies [3, 6, 13, 18] suggest this treatment regimen to be more than sufficient to develop tolerance to the sedative effects of benzodiazepines. No discrimination training or test trials were administered during this period. On the eleventh, twelfth and thirteenth days, animals were injected with the appropriate dose of either diazepam, chlordiazepoxide or solvent and tested for antagonism of the PTZ discriminative stimulus as described for the acute study. To maintain tolerance for these days the rats were injected daily with the appropriate drug four hours after each operant session so that the chronic dosing schedule was maintained.

RESULTS AND DISCUSSION

As previously described [14–17] the rats reliably learned to emit differential responding based upon the PTZ-saline discrimination. It was previously reported that doses of PTZ forming the discriminative stimulus do not affect the rate of responding [16–17]. Rather the discrimination is shown entirely by differential lever selection [17]. It was also established that PTZ stimulus does not generalize to benzodiazepines [15] and that the behaviorally stimulant or depressant effects of drugs do not affect the discriminative stimulus strength [9].

In this experiment all of the animals pretreated either acutely or chronically with the solvent selected the PTZ lever following a PTZ injection (Table 1). Both diazepam and chlordiazepoxide, antagonized the PTZ lever selection and the rats pretreated with the benzodiazepines selected the saline lever after PTZ injection. The potency of either diazepam or chlordiazepoxide in causing PTZ injected rats to select the saline lever was not effected by their chronic administration even though the chronic administration was more than sufficient [3, 6, 13, 16] to produce tolerance to the depressant actions of these drugs. The present data are consistent with previous findings [5, 8, 11] that show lack of tolerance to prevention of PTZ-induced convulsions by diazepam. Lack of tolerance development to the anticonvulsant action of chlordiazepoxide has not been studied. Our data suggest that tolerance may not develop to this action of chlordiazepoxide also since tolerance did not develop to the antagonism of the PTZ stimulus by chlordiazepoxide.

The high degree of selectivity of the anti-PTZ action of benzodiazepines has been demonstrated [19]. It has also been suggested that the ability to prevent or antagonize PTZ-induced convulsions in the laboratory animal reflects the anxiolytic property of these drugs in the clinic [7, 11, 12]. Indeed, prevention of PTZ-induced convulsions is widely used as a screening test for anxiolytic agents [7]. The paradigm reported here offers an alternate procedure for anxiolytic drugs as suggested recently [4,9]. The measures of drug discrimination are objective, quantitative, and the lever

selections can be taken as quantal responses to calculate unit effective doses. The drug discrimination paradigm may also be more economical, as once trained, the same animals may be employed repeatedly.

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