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The Pentylenetetrazole-Cue Antagonist Actions of Bretazenil (Ro 16-6028) as Compared to Midazolam

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RIJNDERS, H. J., T. U. C. JÄRBE AND J. L. SLANGEN. *The pentylenetetrazole-cue antagonist actions of bretazenil (Ro 16-6028) as compared to midazolam.* PHARMACOL BIOCHEM BEHAV **39**(1) 129–132, 1991.—In order to compare the potencies of bretazenil (Ro 16-6028) and midazolam (MDZ) to antagonize the pentylenetetrazole (PTZ) cue, rats were trained to discriminate between 15 mg/kg IP PTZ and saline (FR10, food reinforced). Additionally, other rats were trained to discriminate between 1.0 mg/kg IP MDZ and saline in order to investigate the degree of generalization of bretazenil to MDZ, and to test for the antagonizing effects of PTZ. Both bretazenil and MDZ were able to block the PTZ cue. Bretazenil was about 60 times more potent than MDZ in this respect. In tests for response generalization, bretazenil substituted for the MDZ cue. Bretazenil did not show MDZ-antagonist actions. PTZ did block the MDZ cue and the generalization of bretazenil in the MDZ-trained animals. Assuming that the drug discriminative stimulus functions of PTZ are closely related to its anxiogenic effects, it was concluded that bretazenil may possess powerful anxiolytic properties. Bretazenil did not suppress the response rates which is consistent with previous studies reporting a lack of sedative and muscle-relaxant effects of bretazenil.

Drug discrimination Pentylenetetrazole Bretazenil Midazolam Benzodiazepine Generalization Antagonism Rats

THERE is ample evidence that benzodiazepines can act effectively as discriminative stimuli in rats (2, 14, 15, 17, 19). These studies have demonstrated that the response control exerted by one benzodiazepine will generalise to other compounds from this class. In addition, the stimulus control of benzodiazepines can be blocked by flumazenil and CGS 8216 indicating an effect mediated by the benzodiazepine binding site. At this receptor site, benzodiazepines are believed to affect GABA-ergic activity which is assumed to be responsible for the actual behavioral effects of these drugs. Benzodiazepine agonists have been shown to possess anxiolytic properties in rat models as well as in men (9,13). However, compounds of this class also have sedative and muscle-relaxant effects which are often regarded as disadvantages when such compounds are applied clinically as anxiolytics. The development of benzodiazepine binding techniques has led to the discovery of compounds of both nonbenzodiazepine and benzodiazepine-like chemical structures but, nevertheless, exhibiting apparent anxiolytic activity in addition to a reduced incidence of activity related to sedation and relaxation. Among these drugs are CGS 9896 and bretazenil (Ro 16-6028). The imidazo-diazepinone bretazenil, described as a partial benzodiazepine agonist/antagonist, has been shown to disclose response generalization to the response associated with the chlordiazepoxide cue in a drug discrimination paradigm (15). Additionally, this compound was very potent in blocking pentylenetetrazole-induced tonic convulsions (11). Pentylenetetrazole (PTZ) is a well known and widely used convulsant agent with purportedly anxiogenic effects (1, 5, 9). Additionally, PTZ possesses discriminative stimulus properties at subconvulsive doses (18). Furthermore, it has been suggested for several reasons that the PTZ cue may be closely related to a state of anxiety (4, 5, 18). This assertion is further supported by the finding that anxiolytic compounds, including benzodiazepines, antagonize the discriminative stimulus properties of PTZ (5, 10, 18). In this study we investigated the ability of bretazenil to block the PTZ cue as compared to the cue produced by midazolam, a classical though relatively short-acting benzodiazepine (19). Additionally, bretazenil was tested for substitution in rats trained to discriminate between midazolam and saline.

METHOD

One part of the experiment was carried out in Utrecht, Holland, the other part was carried out in Uppsala, Sweden. In consequence, animals and apparatus in the two laboratories differed

slightly, and will, therefore, be described separately. Training and testing procedures however, were identical in both laboratories.

Animals, Utrecht

Twelve male Wistar rats (CPB-TNO, Zeist, Holland), weighing approximately 250 g at the beginning of the experiment, were individually housed under a nonreversed 12-h light-dark cycle, and a room temperature of 20–22°C. Tap water was freely available. Rats were maintained at approximately 85% of their expected free-feeding weight by giving them 13 g standard laboratory food (Hope Farms, Woerden, Holland), 3 h after each daily session. Friday afternoon, the rats were given 50 g food to last through the weekend.

Apparatus, Utrecht

Six ventilated rat chambers, equipped with two levers, were used. Food rewards were delivered in a tray located at equidistance between the two levers. A detailed description of the apparatus is given by Rijnders et al. (14).

Animals, Uppsala

Seven male Sprague-Dawley rats (ALAN AB, Sollentuna, Sweden), weighing ±400 g at the beginning of the experiment, were used. The animals were housed individually under standard laboratory conditions (temperature 20–22°C; relative humidity 50–60%; a nonreversed 12-h light-dark cycle, lights on at 7 a.m.). The rats were deprived of water in order to maintain their weights at 80 to 85% of their expected free-feeding weights. Food (R3 lab chow, Ewos, Södertalje, Sweden) was freely available in the home cages.

Apparatus, Uppsala

The operant chambers used in Uppsala have been described elsewhere in more detail (8). The chambers contained two response levers separated by a recess in which sweetened water (0.1% saccharin/tap water solution) could be presented. A retractable drinking cup was presented for 4 seconds as a means of delivering the reward.

Drug Discrimination Training

After habituation to the laboratory conditions for one to two weeks, rats were trained to lever-press according to a fixed ratio 10 (FR10) schedule of reinforcement. Thereafter, daily drug discrimination training was started. The rats had to discriminate between the effects of 1.0 mg/kg intraperitoneally (IP) administered MDZ and physiological saline (the Utrecht group), or between the effects of 15.0 mg/kg IP administered PTZ and physiological saline (the Uppsala group). Reinforcements were obtained by pressing the drug-appropriate (D) lever after application of MDZ/PTZ, or the saline-appropriate (S) lever after saline injections. The position of the D and S lever was counterbalanced across rats. The D and S sessions lasted 15 minutes and were basically given according to a 2-weekly alternating sequence: S-D-S-S-D, D-S-D-D-S. The order in which groups were trained was alternated across consecutive sessions as to avoid the possible influence of odour cues (6).

Drug Discrimination Testing

Appropriate lever selection was defined by the accumulation of ten responses on the lever appropriate for the injection condition, with less than five responses on the inappropriate lever. After an animal made nine out of ten appropriate lever selections, and also given that the last three selections were correct, weekly or two-weekly test sessions were started to test for response-generalization and antagonism. The sequence by which different doses of MDZ or PTZ, or any other drug that was tested were given, was counterbalanced across rats. All test sessions were terminated after 6 reinforcements or after 15 min, whichever occurred first. During test sessions, reinforcements could be obtained by pressing either lever according to a FR10 schedule of reinforcement. Test sessions were carried out only when responding during at least the two preceding training sessions had been correct.

Drugs

Midazolam maleate and bretazenil (Ro 16-6028: tertbutyl (s)-8-bromo-11,12,13-,13a-tetrahydro-9-oxo-9h-imidazo(1,5-a)-pyrrolo(2,1-c(1,4)benzodiazepine-1-carboxylate) were gifts from Hoffmann-La Roche (Basel, Switzerland). Pentylenetetrazole HCl was purchased from Sigma Chemicals, St. Louis, MO, and from OPG, Utrecht, The Netherlands. Except for bretazenil, which was suspended in a saline plus tween-80 solution (96 and 4% respectively), all compounds were dissolved in 0.9% isotonic saline. Midazolam was injected in a volume of 2.0 ml/kg, PTZ and bretazenil were injected in a volume of 1.0 ml/kg. Injections were given intraperitoneally, 15 minutes before testing and training.

Data Analysis

The results of the DDL experiments are presented as 1) average percentage of responses on the drug associated lever out of the total number of responses emitted during a test session; the ED_{50} values and slopes of the regression lines were calculated where possible; and 2) response rates expressed as the total number of responses emitted per minute; the effects of drug treatment on response rates were analyzed by means of one-way ANOVA's.

RESULTS

The discriminative stimulus control of responses associated with the training drugs were dose-dependent in both groups, and reached a level of 98.2% MDZ lever responding in the MDZ group (Fig. 1), and 100% PTZ lever responding in the PTZ group (Fig. 2) at the respective training doses. Slopes of the regression lines and ED_{50} values are presented in Table 1.

In the MDZ-trained animals, bretazenil substituted for MDZ in a dose-dependent manner. The maximum level of MDZ-appropriate responding occurred at the dose of 1.0 mg/kg (91.2% MDZ-appropriate responding). The dose-generalization curve of bretazenil was flatter than the one for MDZ; the ED_{50} was about 10 times lower (see Table 1). The MDZ cue was dose-dependently antagonized by PTZ, reducing the level of MDZ responding to 29.4% when MDZ was tested in combination with a dose of 40 mg/kg of PTZ.

In the PTZ-trained group, both bretazenil and MDZ antagonized the PTZ cue dose-dependently and completely. However, the ED_{50} of bretazenil was more than 60 times lower than the ED_{50} of MDZ in regard to antagonizing the PTZ cue (see Table 1). Furthermore, substitution by bretazenil for MDZ in the MDZ-trained rats was decreased when 20 mg/kg PTZ was coadministered with different doses of bretazenil in the MDZ-trained rats (see Table 2).

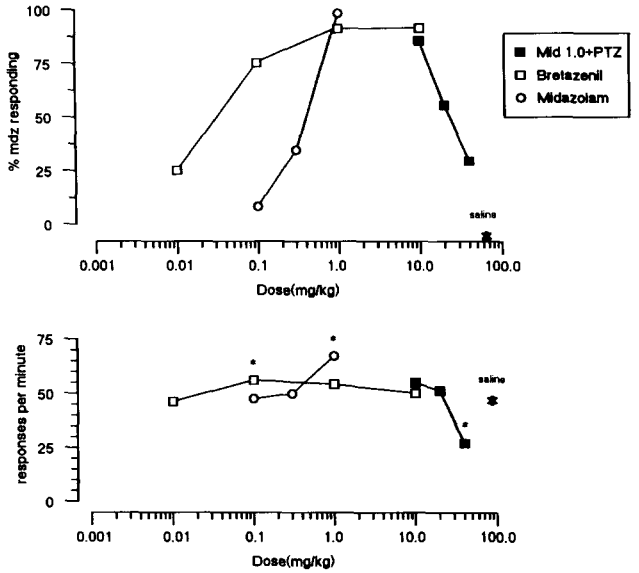

FIG. 1. Results for the generalization tests with MDZ (○) and bretazenil (□) and the antagonist tests with PTZ + 1.0 mg/kg MDZ (■) for the MDZ-trained group. The upper panel depicts the percentages of MDZ appropriate responding as a function of dose. See Table 1 for ED_{50} values and slopes. The lower panel depicts the total number of responses per minute as a function of dose. The asterisks refer to a significant deviation from the saline condition (see text).

To test for possible antagonist actions of bretazenil in the MDZ-trained group, different doses of bretazenil were administered in combination with the training dose of 1.0 mg/kg MDZ.

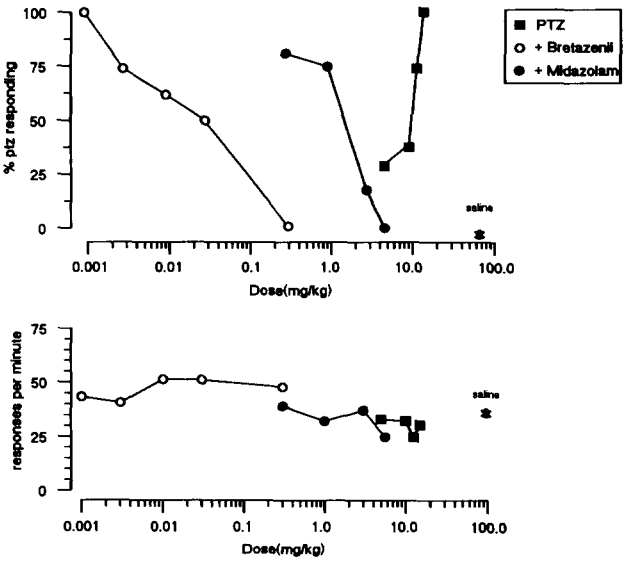

FIG. 2. Results for the generalization tests with PTZ (■), and antagonism tests with bretazenil + 15.0 mg/kg PTZ (○), and MDZ + 15 mg/kg PTZ (●) for the PTZ-trained group. The upper panel depicts the percentages of PTZ appropriate responding as a function of dose. See Table 2 for ED_{50} values and slopes. The lower panel depicts the total number of responses per minute as a function of dose.

TABLE 1

ED_{50} VALUES AND SLOPES OF THE REGRESSION LINES FOR THE DATA PRESENTED IN FIG. 1 AND IN FIG. 2

Drugs	ED_{50}	Slope
MDZ	0.34	90.5
Bretazenil	0.038	33.1
MDZ 1.0 + PTZ	23.7	−93.3
PTZ	8.32	136.3
PTZ 15 + MDZ	1.21	−67.2
PTZ 15 + bretazenil	0.019	−37.3

The first three ED_{50} values and slopes relate to the curves for the upper panel of Fig. 1 (MDZ-trained group). The last three ED_{50} values and slopes relate to the curves for the upper panel of Fig. 2 (PTZ-trained group).

The results revealed no blocking actions of bretazenil at the doses tested (0.01, 0.1 and 1.0 mg/kg; see Table 2).

Response rates are shown in the lower panels of the Figs. 1 and 2, and in Table 2. PTZ in combination with the training dose of MDZ suppressed the response rates dose-dependently as compared to the saline condition, $F(3,33) = 10.8$, $p<0.05$. Additionally, at the highest dose of PTZ tested in combination with 1.0 mg/kg MDZ, two animals failed to select either lever during these tests of 15 min duration. There was a significant dose-dependent incremental effect of MDZ on the response rates in the MDZ-trained group, $F(3,33) = 10.8$, $p<0.05$. When bretazenil was tested for substitution in the MDZ-trained group it also affected the response rates, $F(4,44) = 3.40$, $p<0.05$; however, this effect was biphasic as revealed by the planned comparison analysis which yielded a significant increase of the response rates as compared to the saline condition at a dose of 0.1 mg/kg bretazenil only.

In the PTZ-trained group, response rates were slightly altered by different doses of PTZ, bretazenil and MDZ, although the effects were not statistically significant.

DISCUSSION

Bretazenil substituted completely and dose-dependently for MDZ. Sanger (15) reported similar actions of bretazenil for rats trained to discriminate the "classical" benzodiazepine chlordiazepoxide from saline. These findings suggest that the discrimi-

TABLE 2

PERCENTAGES OF DRUG LEVER RESPONDING (%DLR) AND RESPONSE RATES (RES. RATES) OF ANTAGONIST TESTS IN THE MDZ-TRAINED GROUP

Drugs and Doses (mg/kg)	% DLR	Res. Rates
PTZ 20 + bretazenil 0.1	10.5	38.4
PTZ 20 + bretazenil 1.0	9.3	40.2
PTZ 20 + bretazenil 10.0	39.8	39.4
Bretazenil 0.01 + MDZ 1.0	97.6	59.7
Bretazenil 0.1 + MDZ 1.0	99.8	57.5
Bretazenil 1.0 + MDZ 1.0	92.8	60.5

The generalization of bretazenil to MDZ (as shown in Fig. 1) was decreased when PTZ was coadministered. Bretazenil did not antagonize the MDZ cue. Response rates were not significantly affected.

native stimulus properties of bretazenil are similar to those of the traditional benzodiazepines. However, the generalization curves, yielded by the dose-generalization tests of bretazenil and MDZ carried out in the present study, had different slopes. This suggests that the discriminative cues of bretazenil and MDZ may be similar, but not identical. A relatively flat gradient of the bretazenil generalization curve was also reported by Sanger (15).

Sanger (15) and Sanger et al. (16) reported antagonist actions of bretazenil in rats trained to discriminate zolpidem from saline. Bretazenil did not substitute for zolpidem (15). Zolpidem is a nonbenzodiazepine ligand for benzodiazepine receptors and the zolpidem cue has previously been related to its sedative effects such as the ability of this compound to reduce response rates (3). MDZ is more sedating than most traditional benzodiazepines and is used in anaesthesiology (12). Its discriminative stimulus properties might, therefore, resemble those of zolpidem. However, the results of the present study show that, unlike the zolpidem cue, the MDZ cue could not be antagonized by bretazenil, and that bretazenil did substitute for MDZ. Hence, the differential effects of bretazenil in MDZ- and zolpidem-trained rats, suggest that the MDZ and zolpidem cues are different, and that the discriminative stimulus properties of MDZ do not seem to be related to its sedative effects.

The PTZ-antagonizing effect of MDZ as revealed by this study is consistent with previous reports involving benzodiazepines and PTZ (9). Thus benzodiazepines generally are found capable of blocking the effects of PTZ. However, bretazenil was a more potent antagonist of the PTZ cue than MDZ, which may indicate quantitative differences between the bretazenil and MDZ cues. The antagonism by PTZ and bretazenil was reciprocal as evinced by the finding that the generalization of bretazenil to the midazolam cue was decreased when bretazenil was administered in combination with 20 mg/kg PTZ. The present study also revealed bidirectional antagonist actions of PTZ and MDZ.

Assuming that the PTZ stimulus properties are closely related to its anxiogenic actions, these findings suggest potent anxiolytic activity of bretazenil. Emmett-Oglesby et al. (5) have done extensive research on benzodiazepine withdrawal using PTZ-saline discrimination as a model. Rats were trained to discriminate PTZ from saline and were then treated repeatedly with high doses of diazepam. When the effects of diazepam were blocked by the benzodiazepine antagonist flumazenil, this gave rise to PTZ-appropriate responding. The efficiency of bretazenil to block the PTZ cue as revealed by the present study, and the lack of flumazenil to precipitate withdrawal after prolonged and massive overloading with bretazenil, as reported by Haefely (7), make this drug an interesting candidate to be tested in the withdrawal model developed by Emmett-Oglesby et al. (4,5).

To conclude, the present findings suggest that the MDZ and bretazenil cues are similar but not identical. The results also indicate that the MDZ cue differs qualitatively from the cue produced by zolpidem, as reported by Sanger (15) and Sanger et al. (16). Finally, bretazenil disclosed very potent PTZ-antagonizing actions, which may imply a potent anxiolytic effect of bretazenil at doses that did not reduce the response rates.

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