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The Long-Term Effects of Diazepam and Pentylenetetrazol on the Potentiated Startle Response

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HIJZEN, T. H., F. WOUDENBERG AND J. L. SLANGEN. *The long-term effects of diazepam and pentylenetetrazol on the potentiated startle response.* PHARMACOL BIOCHEM BEHAV **36**(1) 35–38, 1990.—Stress desensitization is observed as a decrease in the disruptive effects of a stressor on behavior when the organism is repeatedly exposed to the stressor. For instrumental behavior, stress desensitization was also reported for rats preexposed to anxiogenic drugs; stress sensitization was reported for rats preexposed to an anxiolytic compound. The present study investigated whether similar effects are found in a noninstrumental task situation. First, rats received 12 daily injections of pentylenetetrazol (PTZ, 20 mg/kg, IP), diazepam (DZP, 5 mg/kg, IP) or saline. After each injection the effect of the drugs on the acoustic startle reflex was measured. No drugs were given during the remainder of the experiment. Eight days later rats were given 5 days of Pavlovian fear conditioning to establish a light as a shock signal. During the next 3 days, potentiation of the startle response by the light was measured. None of the drug treatments had an effect on startle potentiation, indicating that stress sensitivity is not affected by previous administration of PTZ and DZP in a noninstrumental task. An explanation for the different effects found for instrumental and noninstrumental tasks is suggested.

Diazepam Pentylenetetrazol Long-term effects Stressor Anxiety Potentiated startle

WHEN the organism is repeatedly exposed to a stressor, stress desensitization or stress inocculation is observed as a decrease in the disruptive effects of the stressor on behavior (7,9). In addition, for instrumental behavior, stress desensitization was reported for rats preexposed repeatedly to an anxiogenic drug (1,2). In contrast, stress sensitization was reported for animals preexposed to an anxiolytic compound (1). These effects were long lasting; stress (de)sensitization was obtained more than two weeks after the last drug administration. For instrumental behavior, it has been suggested that drug-induced loss of reward initiates new response patterns in order to regain access to the reward (10). Therefore, drug-induced stress desensitization may not be mediated by a reduction of anxiety per se, but may be mediated by responses which interfere with the reaction to the external stressor. The development of new patterns of instrumental behavior during drug treatment might constitute an important difference between stress desensitization as a consequence of repeated exposure to an external stressor and stress desensitization as a consequence of exposure to an anxiogenic drug in an instrumental task. Thus, if new response patterns explain the long-term effects of anxiogenic and anxiolytic drugs mentioned above, drug-induced stress (de) sensitization is not to be expected with a noninstrumental task. In the present study, the effects of repeated exposure to the anxiogenic drug pentylenetetrazol (PTZ), the anxiolytic drug diazepam (DZP), and saline on the acoustic startle response were measured. Then, rats were given 5 days of Pavlovian fear conditioning in which a light CS was followed by shock. After this conditioning, the long-term effects of preexposure to PTZ and DZP on the potentiated startle reflex were evaluated. When a stimulus previously associated with an electric shock of moderate intensity is followed by a loud tone instead of the shock, the amplitude of the startle response will be enhanced (potentiated) (4). In contrast to the nonpotentiated startle response, startle potentiation is selectively sensitive to the effects of both anxiogenic and anxiolytic drugs (3, 4, 14). Therefore, long-term effects of PTZ and DZP on startle potentiation can be expected if they are mediated by an effect on anxiety. No effect on potentiated startle is to be expected if coping strategies constitute the mediating factor.

METHOD

Subjects

Thirty-six male rats of an outbred Wistar strain (CPB, Zeist, The Netherlands) weighing 220–230 g at the beginning of the experiment, were housed four to a cage (60 × 35 × 20 cm). Subjects had free access to food and water. Room temperature was 20–22°C. The experiment was conducted during the first half of the nonreversed 12-hr light-dark cycle.

Apparatus

The startle device was a small rigid chamber (20 × 12 × 15 cm) attached to a heavy superstructure of nylatron and aluminum. The chamber was constructed of stainless steel rods with a nylatron top

and connected to the superstructure by small fiberglass plates. A Bruel and Kjaer (Naerum, Denmark) accelerometer (type 4381) was attached to the top of the cage. The charge amplifier (type 2635) was switched in the velocity position. Startle stimuli (9 kHz, 20 msec) were presented through a Motorola piezo electric tweeter situated 12 cm from one side of the cage. The startle device was inside a sound attenuating room (Industrial Acoustics Company, New York) in which a noise of 48 dB was provided by a random white noise generator (type 231R, Peekel, Rotterdam). Tone and noise intensities were measured inside the cage with a Bruel and Kjaer sound level meter (type 2203). For fear conditioning, a Campden conditioning cage (25 × 23 × 26 cm) was used. Shocks were presented through the grid floor by way of a scrambler and constant current source (Campden, 521 S and 521 C). The cage was placed inside the sound-attenuating room. The startle device and the conditioning room were equipped with two 24 V, 50 mA, AC incandescent signal lights located at the top of the cages.

Tests were controlled by an Apple IIe computer with a GEN-65 function generator interface from Northwest Instruments, Beaverton, OR. Startle amplitudes were sampled each msec and the maximum value within 200 msec after stimulus presentation was obtained.

Procedure

Matching. Six days and again four days before the beginning of the experiment eight 100 dB and eight 105 dB startle stimuli were presented to all animals. These startle amplitudes were used to subdivide the animals into three groups with similar mean startle amplitude and similar startle variance.

Phase 1. During the first 12 days of the experiment the daily sessions lasted 25 min. After an adaptation period of 5 min, 20 startle stimuli (10 at 100 dB and 10 at 105 dB), were presented with an interstimulus interval of 45–75 sec. DZP (5 mg/kg, IP) was administered to one group of rats 25 min before each of the sessions in a volume of 1 ml/kg. PTZ (20 mg/kg, IP) was administered to the second group 10 min before the sessions in a volume of 2 ml/kg. The third group was injected with isotonic saline (0.9% NaCl, IP). DZP (OPG, Utrecht, The Netherlands) was dissolved in a vehicle containing ethyl alcohol, benzyl alcohol, propylene glycol and distilled water. PTZ (OPG, Utrecht, The Netherlands) was dissolved in distilled water. At the end of phase 1, all drug injections were suspended for the remainder of the experiment.

Phase 2. Eight days after the end of phase 1, rats underwent 5 days of Pavlovian fear conditioning. Each of these 25-min sessions started with a five-min adaptation period followed by the presentation of six light (CS) -shock (UCS) pairings. Shock intensity was 0.6 mA and shock duration 500 msec. The light stimulus was presented 3.2 sec before shock onset and lasted 3.7 sec. The intertrial interval was random between 2.5 and 4.5 min.

Phase 3. Fear conditioning was followed 24 hours later by three daily test sessions. Each session started with a 5-min adaptation period followed by ten blocks of four startle trials. Each block consisted of two 9 kHz tones of 100 dB and two of 105 dB. The tone duration was 20 msec. Half the tones were preceded 3.2 sec by the 3.7 sec light. The order of presentation within each block was randomized. The interval between startle stimuli was 45–75 sec. Background noise was 48 dB.

Statistics.

Phase 1. The data of the first 12 days of the experiment were analysed by analysis of variance with Drugs as a between factor having 3 levels (PTZ, DZP, vehicle), and trials as a within factor having 12 levels (trials 1–12). A MANOVA approach for analyzing repeated measurement designs was used in all analyses.

Phase 3. The data on potentiated startle were analysed by a MANOVA, with Drugs as a between factor having 3 levels, Days as a within factor having 3 levels (days 1–3) and CS as a within factor with 2 levels (L+ = light-startle; L− = darkness-startle).

If a main or interaction effect was significant, data were further analysed by comparisons among the means. A 5% level of significance was accepted.

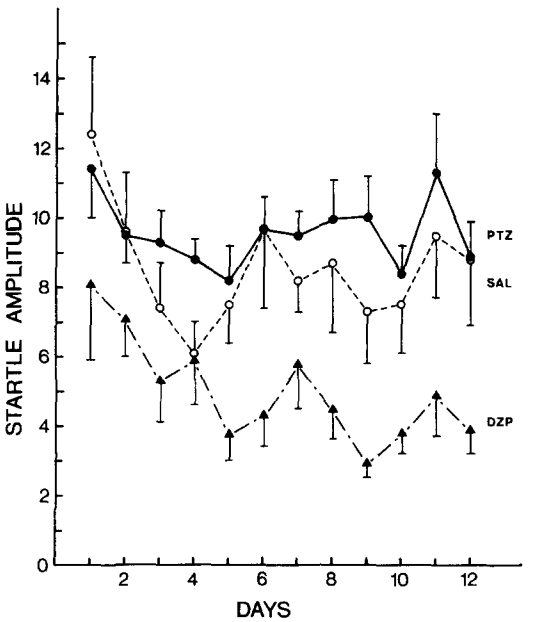

FIG. 1. Mean startle amplitudes as a function of days of drug administration. PTZ = pentylenetetrazol (closed circles); SAL = saline (open circles); DZP = diazepam (triangles). Vertical lines = standard errors of the means. Startle amplitudes in arbitrary units.

RESULTS

Figure 1 shows the effects of PTZ, DZP and vehicle on startle amplitudes during the first twelve days of the experiment. Startle amplitudes were significantly different between drug conditions, $F(2,33) = 5.3$, $p<0.05$. Post hoc comparisons of drug conditions indicated that DZP significantly lowered startle amplitudes from responding in the saline and PTZ condition (DZP vs. vehicle: $p<0.05$; DZP vs. PTZ: $p<0.005$). The main effect of days was not significant.

In Fig. 2, the long-term effects of PTZ, DZP and saline on startle potentiation across three test days are presented. Startle potentiation was significantly different between the CS and non-CS conditions, $F(1,33) = 133.3$, $p<0.001$, and across days, $F(2,32) = 81.4$, $p<0.001$. However, the effect of previous drug exposure was not significant. Although the absolute startle amplitudes of the DZP-treated rats were greater than controls (Fig. 2), potentiated nor nonpotentiated amplitudes differed significantly from each other when compared with t-tests ($p>0.10$). More important, the absolute magnitude of the stress-relevant effect, i.e., the difference between potentiated and nonpotentiated startle amplitudes, did not differentiate drug conditions in any respect.

DISCUSSION

It has been reported that stressed rats show increased response suppression after pretreatment with DZP (1). In contrast, reduced suppression has been reported for rats pretreated with the anxiogenic drugs PTZ and yohimbine (1,2). The effects occurred two weeks after the last drug administration. These long-term effects

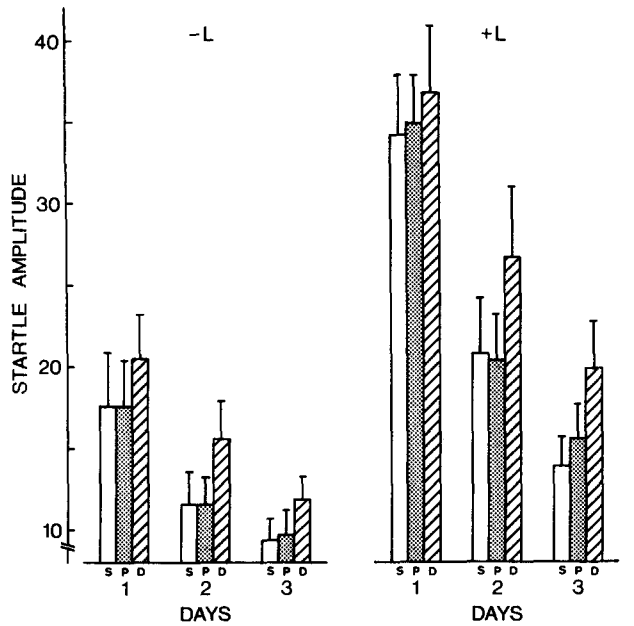

FIG. 2. Mean potentiated (+L) and mean nonpotentiated (−L) startle amplitudes at three successive test days. S = saline; P = pentylenetetrazol; D = diazepam. Vertical lines = standard errors of the means. Startle amplitudes in arbitrary units.

might be mediated by interoceptive cues elicited by the drugs as well as the stressor. However, it also may be assumed that animals develop new response patterns, coping strategies (10,12), in order to obtain sufficient rewards while sedated by DZP. These responses might interfere with performance in later trials in which a stressor but no drug is presented. The startle response used in the present study precluded the development of new responses. Therefore, long-term effects of PTZ and DZP on startle potentiation are to be expected only if the effects are mediated by an altered sensitivity for the anxiety cues associated with the stressor.

Figure 2 shows the effects of shock anticipation on startle potentiation. As expected, mean startle amplitudes were significantly higher in the presence of the light signalling shock than in its absence. Furthermore, the effects of PTZ and DZP on startle potentiation are nowhere different from the effects of the vehicle. Therefore, stress (de)sensitization did not occur after repeated experience with PTZ and DZP, respectively. The results suggest that preexposure to a drug-induced anxiety state has no effect on the anxiety elicited by a stressful external stimulus presented at a later date. The differences between the present results and the findings of Davidson and Lucki (1,2) might be related to differences in experimental task; i.e., noninstrumental vs. instrumental. Figure 1 shows that after DZP administration startle amplitudes remain significantly lower than after administration of PTZ and saline. Apparently, behavioral tolerance did not occur, a result supporting earlier findings (5). In the experiment by Davidson and Lucki DZP suppressed barpressing during the first trials and increased barpress rates significantly in subsequent trials indicating that new response patterns are learned during the execution of the instrumental task. Comparable phenomena have been observed in self-stimulation experiments in which behavioral tolerance and sensitization was found when rats responded while drugged with chlordiazepoxide. When responding and drug administration were dissociated, behavioral tolerance and sensitization did not develop (12,13).

These results are consistent with the idea that animals develop new response patterns when drugs disrupt goal-directed behavior. If these behavioral patterns are triggered in a different situation, an interference with performance may occur.

The disruptive effect of DZP on phase 1 barpressing and the subsequent change in behavior is noticeable in the data reported by Davidson and Lucki [(1), cf. (11)]. However, there is no evidence that the anxiogenic drugs PTZ and yohimbine disrupted instrumental behavior. The effects of yohimbine on phase 1 barpressing are not consistently different from the effects of saline (2). Nevertheless, the doses of PTZ and yohimbine administered by Davidson and Lucki may have affected behavioral patterns not involved in barpressing. Some data seem to support this assumption: Stress desensitization was not observed as a long-term effect of yohimbine when drug administration and barpressing were dissociated. In fact, stress sensitization was found when yohimbine was given in the home cage on nonbarpress days (2). This could mean that when anxiogenic drugs, i.e., yohimbine and PTZ, are administered before barpressing, nonbarpress behavior is disrupted and adaptive behavioral strategies develop which might affect behavior in a different situation [cf. (6)], e.g., facilitate the extinction of a conditioned emotional response (CER) (the test for long-term effects used by Davidson and Lucki). On the other hand, when yohimbine is administered in the home cage, behavioral strategies may develop which retard CER extinction.

In conclusion, PTZ and DZP had no long-term effect on anxiety. The difference between the present results and the results reported by Davidson and Lucki might be related to differences in the experimental task:noninstrumental versus instrumental.

As far as these results are relevant for human behavior, they suggest that the combined effects of behavioral and drug therapies might be contradictory to the long-term objectives of such treatment combinations.

REFERENCES

1. Davidson, T. L.; Lucki, I. The long-term effects of diazepam and pentylenetetrazol on behavioral sensitivity to a stressor. Pharmacol. Biochem. Behav. 27:99–103; 1987.
2. Davidson, T. L.; Lucki, I. Long-term effects of yohimbine on behavioral sensitivity to a stressor. Psychopharmacology (Berlin) 92:34–41; 1987.
3. Davis, M. Diazepam and flurazepam: Effects on conditioned fear as measured with the potentiated startle paradigm. Psychopharmacology (Berlin) 62:1–7; 1979.
4. Davis, M. The potentiated startle response as a measure of conditioned fear and its relevance to the neurobiology of anxiety. In: Simon, P.; Soubrie, P., eds. Animal models of psychiatric disorders. vol. 1. Selected models of anxiety, depression and psychosis. Basel: Karger; 1988:61–97.
5. Davis, M.; Gallager, D. W. Continuous slow release of low levels of diazepam produces tolerance to its depressant and anxiolytic effects on the startle reflex. Eur. J. Pharmacol. 150:23–33; 1988.
6. Demelweek, C.; Goudie, A. J. Behavioral tolerance to amphetamine and other psychostimulants: The case for considering behavioral mechanisms. Psychopharmacology (Berlin) 80:287–307; 1983.
7. Eysenck, H. J. Stress, disease, and personality: The 'inoculation effect.' In: Cooper, C. L., ed. Stress research. Chichester: Wiley; 1983:121–146.
8. File, S. E. Tolerance to the behavioral actions of benzodiazepines. Neurosci. Biobehav. Rev. 9:113–121; 1985.
9. Gray, J. A.; McNaughton, N.; Holt, L.; Tsaltas, E.; Feldon, J.; Shemer, A. The effects of anti-anxiety drugs on tolerance for stress. In: Spiegelstein, M. Y.; Levy, A., eds. Behavioral models and the analysis of drug action. Amsterdam: Elsevier; 1982:175–194.
10. Griffiths, J. W.; Goudie, A. J. Analysis of the role of behavioural

factors in the development of tolerance to the benzodiazepine midazolam. Neuropharmacology 26:201–209; 1987.
11. Herberg, L. J.; Montgomery, A. M. J. Behavioural influences on benzodiazepine tolerance take time to develop. A reply to Goudie. Psychopharmacology (Berlin) 95:548–549; 1988.
12. Herberg, L. J.; Montgomery, A. M. J. Learnt tolerance to sedative effects of chlordiazepoxide on self-stimulation performance, but no tolerance to facilitatory effects after 80 days. Psychopharmacology (Berlin) 93:214–217; 1987.
13. Herberg, L. J.; Williams, S. F. Anti-conflict and depressant effects by GABA agonists and antagonists, benzodiazepines and non-gabaergic anticonvulsants on self-stimulation and locomotor activity. Pharmacol. Biochem. Behav. 19:625–633; 1983.
14. Hijzen, T. H.; Slangen, J. L. Effects of midazolam, DMCM and lindane on potentiated startle in the rat. Psychopharmacology (Berlin) 99:366–370; 1989.