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Chlordiazepoxide Increases the Force of Two Topographically Distinct Operant Responses in Rats

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FOWLER, S. C., R. M. LEWIS, S. E. GRAMLING AND G. L. NAIL. *Chlordiazepoxide increases the force of two topographically distinct operant responses in rats.* PHARMACOL BIOCHEM BEHAV 19(5) 787–790, 1983.—By using operant conditioning techniques one group of 8 rats was trained to reach through a hole in the wall of an operant chamber, and to exert downward responses on a force-sensing circular disk. Eight other rats learned to reach through the hole and grasp and pull toward them a wire bail attached to a force transducer. Both behaviors were maintained on a fixed ratio 20 schedule of water reinforcement. The effects of chlordiazepoxide (CDP, 2.5, 5.0, 10.0 mg/kg) on response force and rate were assessed for both groups. CDP significantly increased response force in a dose-related manner in both groups; regardless of topography, response rate was little affected by the 2.5- and 5.0-mg/kg doses but was decreased by the highest dose. Results were discussed in terms of CDP's antipunishment and neuromuscular effects.

Response force Response rate Chlordiazepoxide Rats

NUMEROUS investigators have demonstrated that low to moderate doses of benzodiazepines increase rates of operant response in rats while higher doses decrease rate [2, 18, 19, 22]; further, such effects appear to be rate dependent [2,19]. Although comparable data for the effects of benzodiazepines on response force are scant, two studies [5,7] do provide some interesting, and perhaps surprising, observations on the effects of chlordiazepoxide (CDP) on operant response force of rats. When these animals underwent extinction following continuous food reinforcement training, 5.0 mg/kg CDP significantly increased response force in comparison to a control group also exposed to extinction [5], and substantial force increases were maintained by the drug group in the face of declining response rate. In a second study [7] rats' responding was reinforced by water on a tandem FR 24 CRFCRFCRFCRF schedule of water reinforcement (that is, 24 unreinforced responses, followed by 4 consecutive, reinforced responses, followed by 24 unreinforced responses, etc.). In this paradigm CDP produced dose-related increases in response force and decreases in response rate with the drug effects occurring primarily during the unreinforced "FR run" (cf., [12]). Because this latter work made repeated observations on the same rats with three different drugs (d-amphetamine, chlorpromazine, and CDP with doses spaced only three days apart), drug interaction effects could not be ruled out as contributing to the CDP-related force increases. Moreover, since both experiments used conditions that arranged for unreinforced responses to occur in close proximity to the relatively "densely" reinforced responding of CRF, one cannot rule out the possibility that the observed increases in force were specific to these two procedures. Accordingly, one purpose of the present research was to examine the effects of CDP on behavior maintained by a conventional schedule of reinforcement (namely FR 20) to extend the generality of the CDP-produced increase in response force. Likewise, response topography was explicitly manipulated as an independent variable in a between-groups design in order to ascertain whether or not a response other than bar-pressing also reflected the force elevating effects of CDP.

An important reason for focusing attention on response force lies in the fact that this dependent variable can provide information about the neuromuscular effects of drugs that is unavailable from the rate measure [7]. In analyzing the effects of CDP on positively reinforced operant behavior in rats, investigators have had difficulty in determining the extent to which the rate-increasing and rate-decreasing effects are produced by any, or some combination, of the many pharmacological effects of this drug. For example, CDP's anxiolytic, anticonvulsant, appetite-enhancing, sedative, ataxic, or muscle-relaxant effects may each have some influence on operant rate increases or decreases. With the discovery of new compounds having primary or exclusive activity on only one of these dimensions (e.g., anxiolytic effects, without sedative or ataxic effects such as CL 218,827 [4,21]), it is important to have behavioral measures which can reflect such pharmacological specificity. Examining response force as a dependent variable in operant behavioral pharmacology is one step in this direction.

METHOD

Animals

Sixteen male, Sprague-Dawley-derived rats with a group

mean body weight of 250 g served as subjects. Rats were maintained on water deprivation in individual home cages that provided continuous access to food. To keep body weight nearly constant, the rats received 3.5 min daily access to water in their home cages after experimental sessions. During the course of the experiment one rat died of undetermined causes.

Apparatus

Two operant chambers measuring 23 cm long, 20 cm wide, and 19 cm high were each enclosed in sound attenuating plywood boxes. The front panels were composed of aluminum; all other sides and the top were made of clear Plexiglas. Flooring consisted of 6.5 mm diameter steel rods running parallel to the front of the chamber. A 6-watt light bulb was centered 4 cm from the top of each chamber to provide illumination. A brass water cup, serviced by a solenoid valve calibrated to deliver 0.1 ml water, was mounted on the lower right front panel. A rectangular opening, 3.0 cm wide and 2.5 cm high, was centered in the front panel 5.5 cm above each grid floor and permitted access to the manipulanda positioned outside the chambers. For one chamber the force sensing manipulandum was an 18 mm-diameter aluminum disk with its center located 25 mm from the outside of the front wall of the chamber. The surface of the disk was parallel to the floor of the operant chamber and was 5 mm above the bottom of the front panel opening through which the subject gained access to the manipulandum. The second chamber was equipped with a "pull-type" manipulandum attached to a Grass Instruments force transducer (FT. 03). The wire bail was triangularly-shaped with the plane of the triangle being parallel to the grid floor and with its 18 mm base positioned 15 mm from the outside of the chamber front wall. The "apex" of the bail was affixed to the shaft of the Grass transducer. Wire, out of which the bail was fashioned, was approximately 1 mm in diameter. An electronic filter with a low-pass corner frequency of 25 Hz was used to reject natural frequency vibrations which occasionally resulted from a "flick" of the rats claws on the pull-type manipulandum.

Contingencies were programmed and data were recorded via a PDP8/e minicomputer and associated peripherals. Details of these techniques are described elsewhere [6]. Under computer control an analog-to-digital converter sampled the analog voltage output of the transducers every 0.01 sec. From these measurements the peak forces of individual responses above an 8-g threshold (cf., [14]) were obtained online. Responses were defined by the force amplitude rising above and then dropping below the threshold. Peak force of response is the maximum amplitude attained during a single response.

Procedure

The rats were randomly divided into two groups of 8 rats each. After two weeks of the water deprivation regimen, the rats were manually shaped to reach through the aperture in the chamber wall to exert forces on the manipulanda. Locating the manipulanda outside the chambers encouraged relatively uniform topographies and precluded biting. In the case of the press-type operandum, the response topography was a downward vertical press, whereas the "pull" rats grasped and pulled the wire bail toward the cage. Thus, force on the press type manipulandum was exerted primarily by forelimb extensors, and pulling forces were mainly applied by forelimb flexion. For both manipulanda the force thresholds and criteria were 8 g. Shaping was much more rapid for the pull than for the press topography. Both groups were given 20 10-min sessions of FR20 training before the first saline injection. Thereafter, doses of CDP were given every 4th day in the following order: 2.5, 5.0, 10.0, 2.5, 5.0, 10.0 mg/kg (expressed as the salt). Each drug day CDP was dissolved in physiological saline and was injected intraperitoneally 30 min before operant sessions. Injection volume was 1.0 ml/kg. Saline control injections were administered the day before each drug day. For each rat data were averaged across replications to provide for split plot, dose-by-topography analysis of variance on the force and rate variables. Because the variances in the forces of the pull group were manyfold larger than those of the press group, the force data were square-root transformed before the analysis of variance computations.

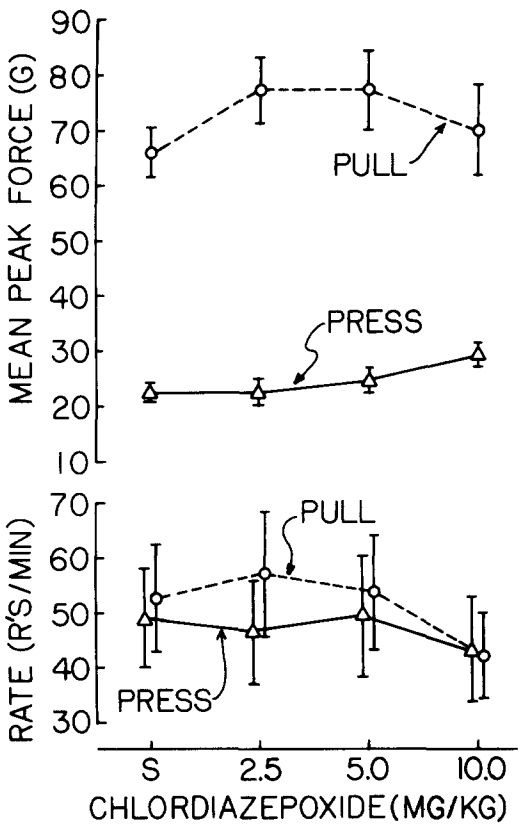

FIG. 1. Mean peak force (upper axes) and average rate of responses (lower axes) as a function of dose of chlordiazepoxide. S indicates saline control performance. Vertical brackets associated with each data point are ±1 S.E.M. Press (n=8) and pull (n=7) refer to the type of operant response topography required of the rat.

RESULTS

Dose-effect data for mean peak force of response and rate of response are shown in Fig. 1. The analysis of variance confirmed the apparent differences in force of response engendered by the two different response topographies, $F(1,13)=111.78$, $p<0.0001$. For both types of topography,

CDP had significant force elevating effects, $F(3,39)=3.14$, $p<0.05$; however, a significant dose-by-topography interaction, $F(3,39)=5.21$, $p<0.01$, substantiates the graphic impression given in Fig. 1 that the dose effect data for response force differ for the two topographies. A series of post hoc comparisons (Tukey's HSD test) showed that mean peak force in the pull group was significantly higher than saline performance only at the 2.5- and 5.0- mg/kg doses; in the press group only the highest dose produced forces significantly higher than saline. Although the dose-effect function for mean peak force of the press rats is quite shallow, our confidence in the reliability of this result is bolstered by data from an unpublished experiment (using procedures similar to those reported here) in which 20 rats received 2.5, 5.0 and 10 mg/kg of CDP, each dose separated by 7 days. Treatment averages for mean peak force (±1 SEM) for saline, 2.5, 5.0, and 10.0 mg/kg CDP were, respectively, 24.1±1.2 g, 26.9±1.3 g, 29.5±1.2 g, 30.8±1.4 g. A repeated-measures analysis of variance applied to these results yielded a significant $F(3,57)=19.355$, $p<0.0001$. These heretofore unpublished data were not combined with those shown in Fig. 1 because the 20 rats had each received a single moderate dose of five different neuroleptics three months before the CDP data were gathered, and the criterion for reinforcement was 16 g not 8 g.

Unlike the force variable, response rate was not significantly affected by type of response topography, $F(1,13)<1.0$, $p>0.10$, but response rate was affected by CDP, $F(3,39)=5.020$, $p<0.01$. For response rate a dose-by-topography interaction did not materialize, $F(3,39)=1.65$, $p>0.10$. By Tukey's HSD test only the highest dose of CDP reduced rate significantly below saline values in the pull group. For the press condition, rates at the highest dose were not significantly different from saline rates by the same post hoc test. However, the shape of the dose effect curve for the press group is probably genuine since rate data from the unpublished experiment cited above were, for saline, 2.5, 5.0, and 10.0 mg/kg CDP, respectively, 61.9±3.9 r's/min, 54.4±4.4 r's/min, 55.4±4.4 r's/min, 40.0±4.6 r's/min. By analysis of variance this dose effect was significant, $F(3,57)=11.971$, $p<0.0001$, and this dose effect on rate parallels the effect shown in Fig. 1.

DISCUSSION

The foregoing results confirm earlier observations indicating that CDP increases operant response force of rats maintained by procedures that do not specifically involve punishment [5,7]. Moreover, the effect is not unique to a single response topography; it can be seen when operant behavior is reinforced on a simple FR20 schedule of reinforcement (in addition to being observed in extinction and tandem FR24 CRFCRFCRFCRF); and CDP-induced force elevations can be observed when response rate is unaffected or decreased. These drug-related changes in response force are probably not nonspecific effects of just any behaviorally-active drug since the neuroleptic chlorpromazine either does not affect response force or decreases it [5,7]; d-amphetamine, in the appropriate dose, may either increase or decrease force of response [7], and the peripherally-acting muscle relaxant, sodium dantrolene, decreases peak force of response [7]. With regard to the specificity question, our unpublished observations for a somewhat different operant setting suggest that selected doses (e.g., 5.0 mg/kg, IP) of pentobarbital may have some modest force elevating effects in rats. It is difficult to account for the force increases observed here in terms of muscle relaxation or ataxia because the doses of CDP were relatively low and response rates were not markedly reduced by the drug. In a behavioral task quite different (non operant) from the one used here, Tilson and Cabe [23] did not observe effects on rats' grip forces until the CDP dose reached 9.0 mg/kg. Additionally, any muscle-relaxant or ataxic effects would appear to be incompatible with the fact that the operant manipulanda were located outside the operant chambers thereby greatly reducing the possibility that responses could be made by the rats' lurching or falling onto the operanda.

For the force variable the significant interaction (note the difference in the shapes of the curves in Fig. 1) between dose and topography may be related to the fact that the pull task requires the rat to use its digits in coordination with forelimb flexion thus making the pull tasks motorically more complex than the press task which does not depend on a grasping response. The highest dose (10 mg/kg, IP; compare with the 9.0 mg/kg in [23] mentioned above) may have begun to affect grasping without affecting overall forelimb force.

The striking difference in force emission between the press and pull topographies is puzzling (see Fig. 1). Even though the force requirement for reinforcement was 8 g for both groups, the pull group needlessly exerted forces three to four times higher than those of the press group. In the absence of an experimental analysis of the pull response we can only speculate on the reasons for this phenomenon. One possible explanation is that the grasping portion of the pull response may provide relatively intense proprioceptive stimulation that elicits the strong flexion in the pull group, whereas the flat, horizontal surface contacted by the press group does not encourage a grasping component, thereby providing less proprioceptive stimulation than the pull response. Another possibility is that the rats learned to put their forelimbs in the correct place, with the peak force being a byproduct of the rapid placing and withdrawing characteristic of FR performance. In a similar vein the pull rats may have learned to pull hard enough to produce discriminable movement in the manipulandum, and comparatively high forces (for a rat) are required to produce appreciable movement with the Grass transducer (1 mm for 200 g). Existing data [14] indicate that rats can, under appropriate conditions, differentiate their forces on a press manipulandum; we have not yet gathered similar data on the pull topography. It is possible that rats in the grasp and pull condition cannot precisely differentiate their force emission.

Both behavioral and physiological hypotheses may be advanced to account for the increased forces occasioned by CDP. First, on the behavioral side, one might suppose that some type of behavioral inhibition was lessened by CDP; such inhibition may be viewed as stemming from "reactive inhibition," i.e., punishing effects related to the exertion and the effort cost of responding [11, 20, 24]. By reducing this kind of inhibition CDP increased the peak force of responses. Dews' [3] concept of attenuation of suppression seems to apply, only if one assumes that unpunished responses are being suppressed by some factor (e.g., reactive inhibition). Likewise, Grays' [9] behavioral inhibition theory of anxiety and antianxiety drugs seems applicable conditional upon the same assumption. These behavioral explanations of CDP's force-elevating effects are unsatisfyingly unparsimonious because they each require the presupposition of an unobservable response-suppressing entity that was not specifically operationalized experimentally. When peak

force is suppressed by punishment, available data [8] suggest that CDP increases peak force.

On the physiological side, several investigators have clearly demonstrated spinal [10, 15, 16, 17] and supraspinal [10, 13, 25] neuromuscular effects of benzodiazepines. However, these studies show that benzodiazepines generally diminish spinal reflexes, probably through enhancement of GABA-mediated presynaptic inhibition. Of the many observed spinal effects of the benzodiazepines only two seem to provide for the possibility of increased peak force of response: these are the benzodiazepine-releated decrease in γ-motoneuron activity [17] and the suppression of Renshaw cell activity [17]. These phenomena invite the speculation that the lowered γ-motoneuron activity could reduce the tone (initial resistance to movement) of the antagonist muscles thereby leading to greater force output in the contracting muscles performing the operant response; and in the face of Renshaw suppression α-motoneurons would be expected to have enhanced output. But in view of the plethora of synaptic influences on the α-motoneurons and considering the difficulty of generalizing results from anesthetized or spinal animals to unanesthetized, intact animals, such speculation demands skepticism.

Whether or not the CDP-induced increase in rats' peak force of response is a manifestation of either the anxiolytic-antipunishment or neuromuscular effects (or some combination of these) of benzodiazepines cannot be ascertained without further work with intact animals. In addition to extending the current observations to other benzodiazepines, it would seem worthwhile to attempt to develop evidence that the CDP-force effect in rats is benzodiazepine specific by making use of the antagonist, RO 15-1788 [1,21] and by examining systematically the effects of pentobarbital on peak force. Moreover, the compound CL 218,827 [4] could be of use in assessing the extent to which the CDP-force effect is possibly related to anxiolytic-antipunishment and neuromuscular factors.

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