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BRIEF COMMUNICATION

# Conditional Attenuation of the Antipentylenetetrazol Activity of Chlordiazepoxide

HARALD K. TAUKULIS

*Florence M. Christie Laboratory for Life Sciences, University of New Brunswick, Saint John, New Brunswick E2L 4L5, Canada*

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TAUKULIS, H. K. *Conditional attenuation of the antipentylenetetrazol activity of chlordiazepoxide.* PHARMACOL BIOCHEM BEHAV **46**(2) 467–471, 1993. – In a study of drug-drug conditioning, chlordiazepoxide (CDP) was administered to rats 30 min before chlorpromazine (CPZ) every 3–4 days for 5 weeks. Thereafter, CDP was tested for its ability to a) increase animals' exploration of the normally avoided "exposed" arms of a plus-maze (a screening method for anxiolytic substances) and b) protect against seizures induced by pentylenetetrazol (PTZ). The CDP–CPZ conditioning procedure potentiated the usual increase in open-arm activity seen in CDP-injected rats but attenuated the anti-PTZ effect. When injected with CDP 60 min prior to PTZ, rats with a previous history of CDP–CPZ pairings had shorter latencies to seizure onset and showed a greater likelihood of progression to the most severe behavioral indices of a maximal seizure relative to control subjects with a history of backward drug pairings (CPZ–CDP) or pairings of CDP with isotonic saline.

Chlordiazepoxide Chlorpromazine Plus-maze Conditional interaction Seizures Anxiolytic Anticonvulsant Pentylenetetrazol

MANY benzodiazepines (BDZs) have both anxiolytic and anticonvulsant properties, and both effects may be mediated by the drugs' actions on GABAergic systems (5,8). BDZs are known to facilitate GABAergic inhibition, presumably through an allosteric modulation of the GABA–BDZ–chloride ionophore receptor complex (6). Some other classes of drugs with anxiolytic and anticonvulsant effects, like sodium pentobarbital, also potentiate GABAergic transmission (6,11,18).

In an animal model of anxiolysis, the elevated plus-maze, BDZs will increase the amount of time rats or mice will leave two arms enclosed by high walls and explore two "exposed" arms unprotected by walls (10). In this model, the effect of at least one BDZ, diazepam, can be potentiated as a function of prior "drug-drug" conditioning. For example, Taukulis and Brake (15) injected rats with diazepam and then, 30 min later, injected chlorpromazine (CPZ), a phenothiazine neuroleptic. This procedure was repeated on 12 occasions spaced 7 days or more apart. In a test trial, animals were given a single injection of diazepam and placed into the plus-maze. When compared with appropriate controls that had not experienced diazepam–chlorpromazine pairings but were injected with diazepam prior to the plus-maze test, experimental animals spent substantially more time in the open arms and entered them more frequently. This effect was also obtained when diazepam was paired with haloperidol, a butyrophenone neuroleptic (16), or yohimbine, an $\alpha_2$-adrenoreceptor antagonist (Taukulis, unpublished).

The neurophysiological basis for this effect is not yet known, but evidence of complex, reciprocal interactions between dopaminergic and GABAergic systems (7,13) led to the suggestion that a conditional potentiation of BDZ activity by dopamine blockers like chlorpromazine may stem from a temporary facilitation of GABAergic neurotransmission (16). If this hypothesis is correct, and if a BDZ's anticonvulsant effect also depends upon its influence on GABAergic neurons, then a potentiation of its capacity to prevent or attenuate certain chemically induced seizures might also be detectable following BDZ–neuroleptic conditioning. The present experiment was designed to evaluate this possibility. Rats were given 10 pairings of chlordiazepoxide (CDP) with CPZ during a conditioning phase. In a subsequent test phase, the effect of CDP on plus-maze activity was determined, followed by an assessment

of this BDZ's effects on seizures induced by pentylenetetrazol (PTZ).

## METHOD

### *Subjects*

Thirty-six Long-Evans male rats (Charles River, St. Constant, Quebec) were used as subjects. They weighed between 236–309 g (average = 272 g) at the beginning of the experiment. All were housed in standard steel cages in a room maintained at 22–24°C with a 14 L : 10 D cycle. Water and Purina Rat Chow were available in these cages at all times.

### *Apparatus*

*Plus-maze.* The maze was constructed of wood coated with several layers of black polyurethane. Two opposing arms (the "open arms") were boards, each 50.0 cm long and 10.0 cm wide, bordered with a 1.5-cm high lip designed to minimize the chances that an animal would slip from an arm to the floor. The other two opposing arms (the "enclosed arms") were the same dimensions as the former but were bordered with 40.0-cm high walls. At the juncture of the four arms, there was a 10.0-cm square neutral zone upon which an animal was placed at the beginning of a trial. The entire maze was elevated on a free-standing platform to a height of 82.0 cm. The maze was housed in a room adjacent to the animal holding quarters. Ceiling lighting was adjusted to maintain a brightness averaging 155–165 lux at the ends of the open arms. A Sony (CCD-V9) videocamera was mounted above the maze to record locomotor activity.

*PTZ test chamber.* For the PTZ seizure test, each animal was placed into a covered glass enclosure measuring 19.0 × 25.0 × 45.0 cm. The glass floor of this enclosure was layered with wood chips to a depth of 2.0 cm.

### *Drugs*

Chlordiazepoxide HCl (Roche Laboratories, Nutley, NJ) and chlorpromazine HCl (Sigma Chemical Co., St. Louis, MO) were dissolved in isotonic saline to concentrations of 4.0 and 5.0 mg/ml, respectively. All injections, including control injections of isotonic saline, were administered at a volume of 2.0 ml/kg. Pentylenetetrazol (Sigma) was dissolved in isotonic saline to a concentration of 100.0 mg/ml and administered in a volume of 1.0 ml/kg.

### *Procedure*

At the beginning of the experiment, 36 rats were divided into three groups ($n$ = 12) equated on the basis of animals' body weights. Over a period of 5 weeks, they were given four pairs of injections per week. On Tuesdays and Fridays, the paired injections (IP) consisted of 8.0 mg/kg CDP followed by 10.0 mg/kg CPZ (group CDP-CPZ), CPZ followed by CDP (group CPZ-CDP), or CDP followed by saline (group CDP-SAL). A 30-min interval occurred between injections. On Mondays and Thursdays, the two injections consisted only of isotonic saline. These were included to minimize associations between the injection procedure and the drug effects themselves, thereby reducing potential interference with drug–drug associations. Past experience has shown that this procedure also minimizes struggling and other signs of distress that can occur during the injection process.

At 120–144 h after the last drug pairing, the plus-maze test procedure was performed. Each rat was injected with either 8.0 mg/kg CDP or 2.0 ml/kg isotonic saline and, 25 min thereafter, placed into a glass and steel enclosure with floor dimensions of 61.5 × 72.0 cm. Placement here served the purpose of arousing the animal and increasing subsequent maze exploration. After 5 min, the rat was removed and positioned diagonally on the central square of the plus-maze at the juncture of the open and enclosed arms. The experimenter then left the room while the animal's activity was videotaped for a 5-min period. Forty-eight hours thereafter, a second plus-maze test was performed. It was identical to the first except animals that had received CDP prior to the first test (six from each group) now received saline and those that had previously been injected with saline now received CDP.

Within 72–96 h after its second plus-maze experience, each rat was tested for its susceptibility to PTZ-induced seizure following a protective dose of CDP. Removed from its home cage, it was injected with 8.0 mg/kg CDP and then replaced. Sixty minutes later, it was carried to a separate test room, injected (IP) with 100.0 mg/kg PTZ, and placed into the glass

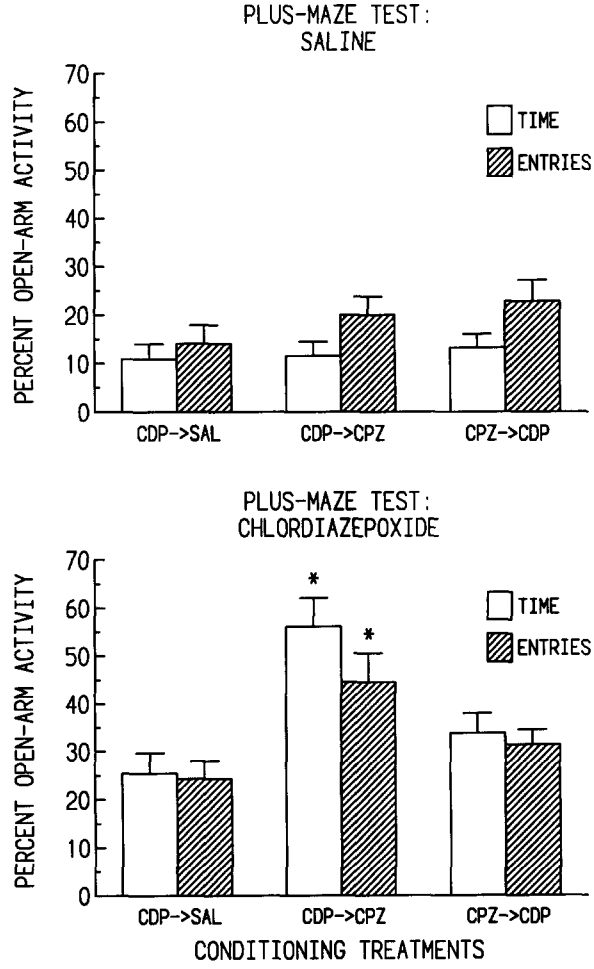


FIG. 1. Effects of a control injection of saline (upper graph) or 8.0 mg/kg chlordiazepoxide (CDP, lower graph) on the mean percentage of time in and entries into the open arms of the plus-maze exhibited by three groups of rats with different conditioning histories. *For both the time and entries measures following CDP, Newman-Keuls analyses revealed that group CDP-chlorpromazine (CPZ) differed significantly from each of the other groups at $p < 0.01$ or less.

test chamber, where its behavior was recorded by an observer naive with regard to the animal's previous conditioning history.

A rat injected with PTZ, when unprotected by an anticonvulsant drug, exhibits a seizure pattern that involves a sequence of several definable phases. The episode normally begins with a series of myoclonic jerks and ends in a phase of tonic/clonic convulsions involving hindlimb extensions, loss of postural control, and uncoordinated movements. Both the first myoclonic jerk and the progression to a maximal seizure are clearly identifiable events and therefore were utilized here as the indices of PTZ activity.

Videotaped activity in the plus-maze was scored by an unbiased observer who counted the number of entries that animals made into open vs. enclosed arms and the number of seconds (of 300) that they spent in each. An entry was counted only after all four of a rat's paws were within the boundaries of an arm immediately following a period during which no paws were within that arm. Similarly, timing was not begun until all four paws were within an arm. Prior to statistical analysis, the time in the open arms was expressed as a percentage of the total time an animal spent in both arms and the number of entries into the open arms was expressed as a percentage of total arm entries. For each measure, a three × two × three analysis of variance (ANOVA) was performed. The factors were: a) groups; b) test treatment (saline vs. CDP); and c) order of testing (saline first vs. CDP first). Where appropriate, Newman-Keuls and correlated *t*-tests were used for pairwise comparisons.

## RESULTS

### *Plus-Maze Test*

The mean percentage of time spent in the open arms by each group and the mean percentage of entries into each arm is shown in Fig. 1. Open-arm activity following both the test injection of saline (upper graph) and the injection of CDP (lower graph) is depicted.

For the time measure, a significant main effect of groups appeared, $F(2, 30) = 5.83$, $p < 0.01$, and an effect of test treatment, $F(1, 30) = 111.03$, $p < 0.001$, as well as an interaction between these two factors, $F(2, 30) = 13.96$, $p < 0.001$. Analysis by the Newman-Keuls method indicated that, following a CDP injection, group CDP-CPZ spent more time in the open arms than either group CPZ-CDP or group CDP-SAL ($p < 0.001$ in both cases; the latter two groups did not differ). No between-group differences appeared following a saline injection. Correlated *t*-tests revealed that all three groups spent more time in the open arms following CDP than following saline ($p < 0.001$ in all cases). The order of testing had no detectable effect either alone or in interaction with either of the other factors.

A similar analysis of open-arm entries revealed that the main effect of groups fell short of significance, $F(2, 30) = 3.23$, $p < 0.06$, but the test treatment factor yielded, $F(1, 30) = 32.72$, $p < 0.001$, and a significant interaction between these two factors emerged, $F(2, 30) = 4.09$, $p < 0.03$. Analysis by the Newman-Keuls method revealed that, following the CDP treatment only, group CDP-CPZ exhibited a greater percentage of open-arm entries than either group CPZ-CDP ($p < 0.01$) or group CDP-SAL ($p < 0.001$; the latter two groups did not differ). Correlated *t*-tests comparing percentage of open-arm entries after CDP with the percentage noted after saline revealed that all three groups entered the open arms more frequently after CDP ($p < 0.05$ in all cases). No effects of order of testing were detected.

As a measure of general activity, the total number of entries into both types of arms was tallied for each group and is shown in Table 1. ANOVA revealed no differences as a function of any of the three factors of interest.

TABLE 1

MEAN NUMBER OF ENTRIES (± SEM) INTO BOTH ARMS OF THE PLUS-MAZE FOLLOWING INJECTION OF SALINE OR CDP

| | Total Arm Entries | |
|---|---|---|
| Groups | Saline Test | CDP Test |
| CDP-SAL | 8.8 ± 0.7 | 9.4 ± 1.1 |
| CDP-CPZ | 10.2 ± 0.7 | 11.7 ± 1.0 |
| CPZ-CDP | 11.2 ± 1.3 | 11.3 ± 1.2 |

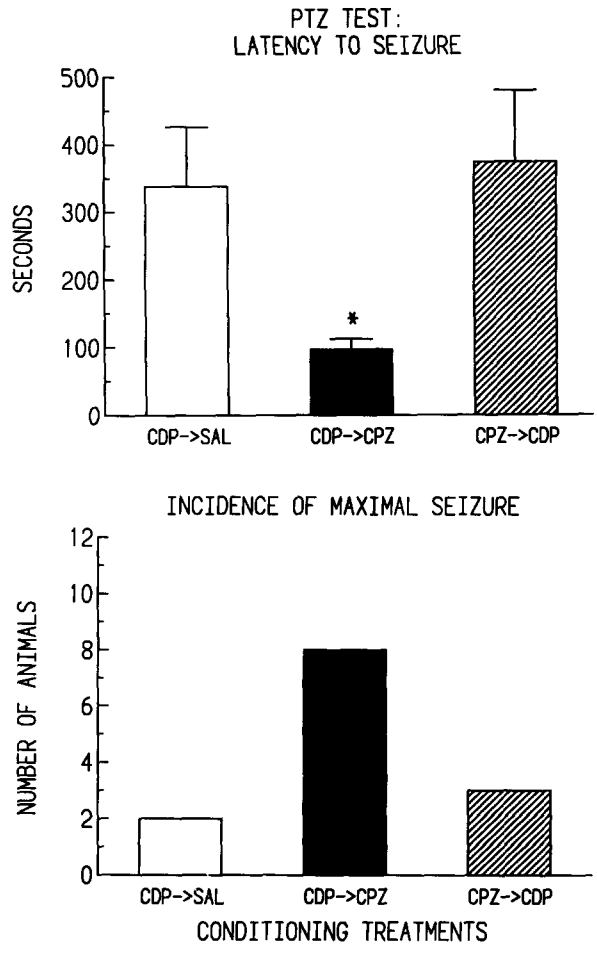


FIG. 2. Top: Effects of 8.0 mg/kg chlordiazepoxide (CDP) on the mean latency to onset of a pentylenetetrazol (PTZ)-induced seizure exhibited by three groups of rats with different conditioning histories. *Significantly different from each of the other groups, $p < 0.05$. Bottom: Incidence of progression to the severest symptoms indicative of a maximal seizure in the three groups ($n = 12$ per group).

*PTZ Seizure Test*

The mean group latencies to the initial myoclonic seizure are shown in Fig. 2 (upper graph). One animal from group CDP-SAL showed no signs of seizure and was not included in the statistical analysis. A one-way ANOVA indicated a main effect of groups, $F(2, 32) = 3.64, p < 0.05$. Subsequent pairwise comparisons revealed that group CDP-CPZ had a shorter mean latency relative to both other groups ($p < 0.05$, Newman-Keuls), while the latter did not differ from one another.

The incidence of progression to a maximal seizure is shown in the lower half of Fig. 2. By Fisher's Exact Test, significantly more animals in group CDP-CPZ showed this effect than those in group CPZ-CDP ($p < 0.05$) or group CDP-SAL ($p < 0.02$).

## DISCUSSION

The pairing of CDP with chlorpromazine resulted in a potentiation of CDP's capacity to increase open-arm activity in the plus-maze, a behavior that is putatively an index of a drug's anxiolytic potential. This is consistent with earlier reports of chlorpromazine's effect on another BDZ, diazepam (15,16). Backward pairings of the two drugs (i.e., CPZ-CDP) did not have the same effect. This suggested that, in the forward pairing group (CDP-CPZ), a process of conditioning occurred through which the cue drug, CDP (or one of its metabolites), acquired conditional properties by virtue of the fact that it signalled the imminent onset of the effects of CPZ. If a pharmacological interaction not involving conditioning were responsible for this effect, then group CPZ-CDP should have shown a similar response to CDP.

Contrary to expectation, CDP-CPZ pairings attenuated rather than potentiated CDP's capacity to protect against PTZ seizures. This was evident in both the decreased latency to the first myoclonic episode shown by group CDP-CPZ and in the greater incidence of maximal seizures found in these animals. This outcome suggests that a conditional facilitation of GABAergic neurotransmission (beyond the unconditional effect usually ascribed to benzodiazepines) is unlikely to be responsible for both the plus-maze and the anti-PTZ effects observed in group CDP-CPZ.

The neurophysiological basis for the plus-maze potentiation effect may reside with one or more subsystems of the serotonin receptor group. Benzodiazepines are known to inhibit serotonergic transmission by way of GABAergic mediation (12,14,17), and inhibition of activity in certain serotonergic subsystems [5-hydroxytryptamine$_{1A}$ (5-HT$_{1A}$), 5-HT$_2$, 5-HT$_3$] is believed to contribute to anxiolysis in humans and putatively analogous effects in animals (2,12,17). Chlorpromazine has a high affinity for 5-HT$_2$ receptors and can reduce 5-HT$_2$ receptor binding when administered once a day for as few as 3 days (1). When a benzodiazepine has been paired with chlorpromazine on several occasions, it may, by virtue of this association, evoke a conditional decline in 5-HT$_2$ receptor activity. Such an effect might account for the plus-maze potentiation because it has been reported that 5-HT$_2$ receptor antagonism by drugs like ritanserin can increase the open-arm exploration exhibited by rats (4,20).

The attenuation of the antiseizure protection normally afforded by CDP is also assumed to be a product of a conditioning process. Dopamine (D$_2$) antagonists like CPZ are proconvulsant in some models of rodent epilepsy (19), and the conditioned response elicited by the CDP cue may be related to this in some way. D$_2$ receptor agonists like lisuride, on the other hand, can have anti-PTZ properties (9), and future research will determine whether these drugs will have an anticonvulsant-potentiating effect when paired with BDZs. In any case, the search for the substrates of the conditional attenuation phenomenon should extend beyond the GABA-BDZ complex. This contention is supported by recent evidence suggesting that the antipentylenetetrazol effect of some BDZs may be linked to GABA-independent BDZ receptor sites (3).

Another avenue to pursue is the possibility that CDP-CPZ pairings bring about conditional changes in CDP pharmacokinetics. For example, accelerated biotransformation may ensure that the peak of the drug's anticonvulsant efficacy has passed by the time the PTZ test is performed. Such an explanation would not necessarily be inconsistent with an enhanced plus-maze effect if the two tests are performed at different post-CDP intervals, as was the case in the present experiment (i.e., 30 min for the plus-maze test and 60 min for the PTZ test). Indeed, it could account for both phenomena if it ensures that larger than usual quantities of CDP or its metabolites are available at the receptor sites mediating the plus-maze effect, while smaller than usual quantities are present at the sites mediating the anti-PTZ effect.

The conditional attenuation of CDP's antipentylenetetrazol action is reliably obtainable. In an experiment similar to the present one, CDP's influence on both the latency to a PTZ seizure and the duration of several phases of the seizure were shown to be affected by prior CDP-CPZ conditioning (A. Veall, unpublished manuscript). No studies have, as yet, been performed to determine how long the conditioned response will persist or what the optimal parameters for conditioning and testing are. The pairing of nonneuroleptic, receptor-specific drugs with BDZs may also assist in the development of hypotheses concerning possible neural substrates of this effect.

Previous studies (15,16) revealed that the anxiolytic, muscle-relaxant, and thermic effects of BDZs are susceptible to modulation by drug-drug conditioning procedures. The present experiment added the anticonvulsant property of these drugs to this list. Further, it illustrated how the drug-drug conditioning paradigm may be fruitfully used to obtain insights into putative drug-neurotransmitter interactions.

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