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# The Mechanism of Anti-muricidal Effects of Chlordiazepoxide[1]

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QUENZER L. F. AND R. S. FELDMAN. *The mechanism of anti-muricidal effects of chlordiazepoxide.* PHARMAC. BIOCHEM. BEHAV. 3(4) 567–571, 1975. – Muricidal activity of rats is suppressed by chlordiazepoxide (CDP). At appropriate doses the CDP effect is reversed by repeated testing, by pretreatment with CDP, and by concomitant dosing with caffeine. This points to the general behavioral depressant action of CDP which undergoes tolerance as being primarily responsible for the anti-muricidal activity of CDP.

Muricide Chlordiazepoxide Caffeine Tolerance

ALTHOUGH anti-aggression properties of chlordiazepoxide (CDP) have been documented [2, 7, 10], conflicting experimental results are common. It is generally assumed that various testing situations elicit different aggressive behaviors having different underlying physiological substrates [17]. Therefore, it is not surprising that CDP exerts differential effects depending upon the aggression paradigm used. Further, the experimental species that is used, and the differences in drug dosages as well as the frequency of administration, have all been shown to be significant factors in determining the anti-aggression effects [4].

Until recently, the importance of behavioral depression for the reduction of aggression after administration of CDP has been determined by a conventional test of locomotor ability such as the inclined screen [8], rotarod [11,12], jiggle box [15] or activity cage [1]. While each of these is an adequate measure of locomotor ability in itself, the differences between the motivational and response parameters of the aggression paradigm and the test of behavioral depression has not been considered. The finding that the depressant action of oxazepam (a drug in the same class as CDP) in a conflict situation disappears after repeated administrations of the drug [16,21] suggests the use of chronic drug administration as a method of eliminating behavioral depression either before or within the aggression paradigm itself.

While mouse-killing by rats is one of the aggressive behaviors that has been suppressed by CDP, recently Wnek *et al.* [25] induced an increase of mouse-killing in rats treated with low doses of CDP. However, even in cases in which higher doses were used and mouse-killing was attenuated, the importance of the behavioral depressant effect for the reduction in aggression was not clear. Horovitz and his coworkers [11,12] and Karli [14] determined that mouse-killing is diminished by benzodiazepines only at doses that produce motor impairment, while Loiselle and Capparell [15] found that CDP-treated animals killed significantly fewer mice at a dose that did not produce ataxia. Both studies, however, determined the drug effect only after acute administration. Therefore, differences in the determination of the role of behavioral depression on muricide activity may only have reflected differences in methods of measuring behavioral depression.

We report here three experiments in an attempt to clarify the role of behavioral depression in the anti-muricidal effects of CDP. In the first experiment we attempted to eliminate CDP-induced depression by chronic administration to allow tolerance to develop to the depressant action of the drug during consecutive muricide tests. In a second experiment we attempted to answer the question of whether tolerance was a physiological or behavioral phenomenon. A third experiment investigated the possibility that a CNS stimulant could reverse CDP suppression of muricide.

## EXPERIMENT 1

In this study, the effect of 8 consecutive daily administrations of CDP to 3 different dose groups on the latency of mouse-killing was determined. It was hypothesized, on the one hand, that if behavioral depression plays a signifi-

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cant part in the reduction of mouse-killing, the latency to kill mice should be highest during initial drug tests. Then as testing continues latency should decrease as the tolerance to the behavioral depressant effect gradually develops. On the other hand, if there were an anti-muricide factor independent of behavioral depression, the latencies of mouse-killing should remain high for the duration of the drug tests.

*Method*

The animals in the first experiment were 32 male Sprague-Dawley rats 4–6 months old and weighing 350–475 g. Of these, 28 completed all tests. They were housed individually and fed and watered ad lib. These animals quickly and reliably killed mice placed in their home cages prior to any drug treatment. During each test session a live mouse was placed in the living cage of each rat and the latency to kill the mouse was recorded. If the mouse was not killed within 15 min it was removed from the cage. Mice killed during the session were removed promptly from the cage to prevent the rat from eating it. After 3 daily no-drug (saline) muricide tests, each rat received CDP IP 30 min before each of 8 successive daily muricide tests. The rats were assigned to 3 dose groups. The animals that completed all the tests were distributed as follows. One group, N = 9, received 25 mg/kg of CDP before each daily muricide test; the second group, N = 10, received 50 mg/kg, and the third group, N = 9, received 75 mg/kg before each test.

*Results and Discussion*

Figure 1 shows the daily percentage of muricidal rats in the 3 dose groups during 3 consecutive saline and 8 CDP test days. The columns represent only those rats that survived all 8 CDP tests. It can be seen that CDP suppresses muricide in all dose groups and a comparison of the percentage of muricide suppression showed that the amount of suppression was 47.2%, 33.7% and 29.2% for the 75, 50 and 25 mg/kg groups respectively, but an analysis of variance for dichotomous data (kill vs no-kill) did not show a significant dose effect [24]. An analysis of variance comparing the latency of muricide, while assigning a latency of 15 minutes to non-killers, also showed no significant dose differences. However, t-tests for paired observations comparing latency on Day 3 (saline) with Day 4 (drug) showed significant differences for all three dose groups (for 25 mg/kg: $t = 2.58$, $p<0.05$; 50 mg/kg: $t = 4.04$, $p<0.01$; 75 mg/kg: $t = 4.15$, $p<0.01$).

Comparing muricide latency over days, an analysis of variance again showed no dose effects, but combining the 3 dose groups and using a conservative test assuming extreme heterogeneity of variance the result showed that latency declined significantly over days, $F(1,8) = 11.96$, $p<0.01$. Also analysis of variance for dichotomous data showed a significant days effect, $F(7,175) = 6.57$, $p<0.001$. These data indicate that CDP suppressed muricide in the three dose groups, and that with continued testing tolerance to the depressive effect of the drug had occurred.

Correlated t-tests that compared latency between Day 3 (saline) and Day 11 (last drug day) showed that there were significant differences for the 25 and 75 mg/kg groups ($t = 3.26$, $p<0.02$, and $t = 2.30$, $p<0.05$, resepctively) but the difference for the 50 mg/kg group was not significant (see Fig. 2). Also for the 50 mg/kg group a t-test for correlated

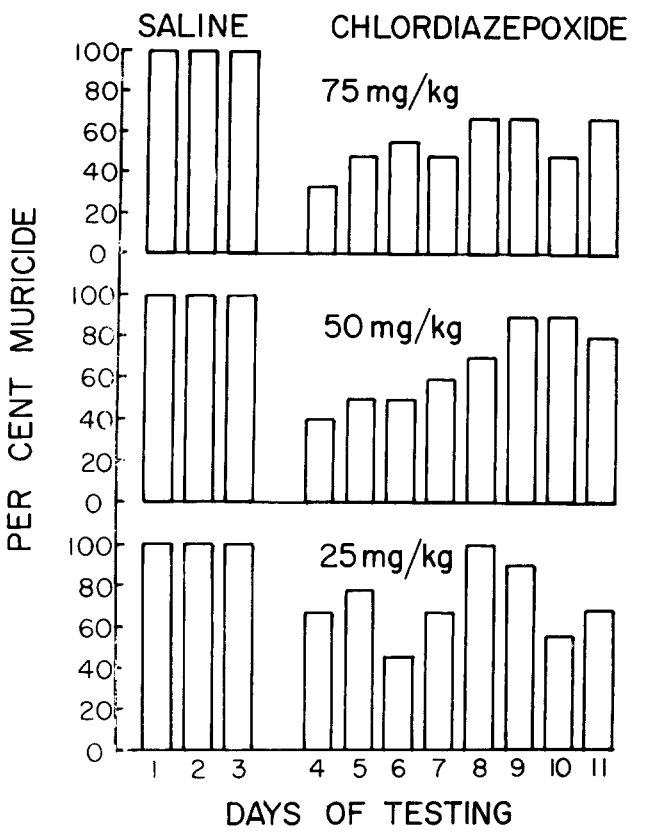


FIG. 1. Comparisons of percentages of muricidal rats under no-drug (saline) and drug (CDP) conditions. Rats had to kill a mouse within 15 min to be designated as muricidal.

measures comparing mean latencies for the first 4 drug days with the last 4 also showed a significant difference ($t = 3.38$, $p<0.01$), but similar comparisons for the 75 and 25 mg/kg groups were not significant.

These data show that for the 50 mg/kg group muricide latency had returned to control levels by the end of the drug testing period. The analysis of variance for dichotomous data also showed that there was a significant Days effect for this group, $F(7,63) = 2.47$, $p<0.05$). These results strongly suggest that a marked tolerance to the antimuricidal effects of CDP had occurred in this group.

The middle graph in Fig. 1 shows the percentage of kills over days for the 50 mg/kg group and illustrates the significant progression toward renewed muricidal activity with continuous testing, presumably as a result of drug tolerance. Further, the analysis of the latency data shown in Fig. 2 showed that by Day 11 muricidal latency returned to levels not significantly different from control levels. The question that naturally arises is why similar effects were not seen in the 25 and 75 mg/kg groups even though the analyses of variance indicated there was some evidence of tolerance in all groups. A reasonable explanation would be that 25 mg/kg was not sufficient to induce microsomal enzyme activity to the extent that tolerance was detectable in muricide tests. Concerning the 75 mg/kg group, there was a strong consistant suppression of muricide but the maximum tolerance that occurred was possibly unable to completely compensate for the depression caused by 75 mg/kg doses.

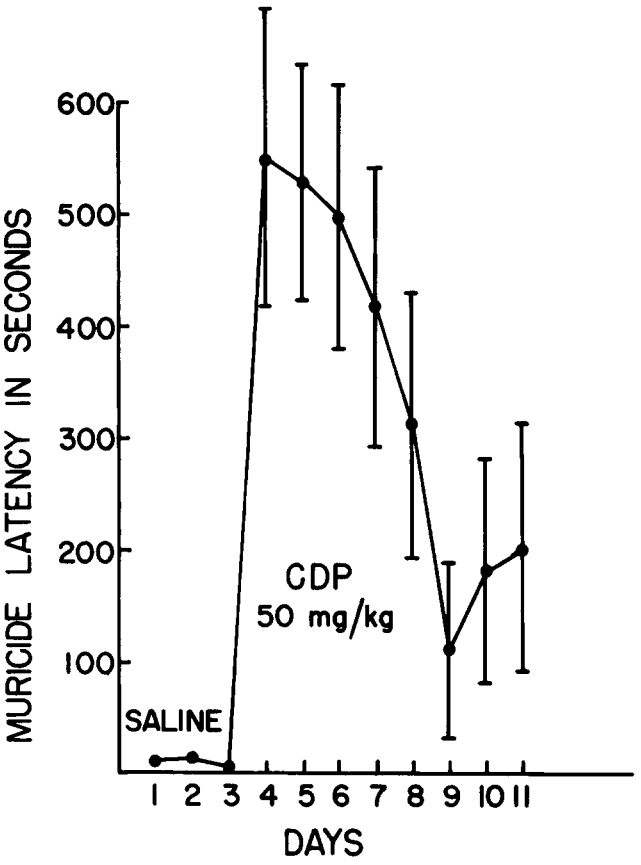


FIG. 2. Latency of muricidal activity under no drug (saline) and drug (CDP) (50 mg/kg) conditions. Non-muricidal rats were assigned a latency of 15 min. The vertical bars through the data points indicate the standard error of the mean.

Moreover, a degree of toxicity probably existed in this dose group because 4 of the 13 rats died during the course of drug testing, and a fifth rat died 2 days after the tests were completed. In the 50 and 25 mg/kg group all rats survived the 8-day drug tests, but one rat in the 50 mg/kg group died 2 days after the drug tests were over. Thus, drug toxicity may have been partly responsible for the failure of the 75 mg/kg group to return to high levels of muricide with continuous testing.

A previous study by Feldman [5] supports the argument about limits of tolerance. Rats were tested in a bright–dark discrimination problem on a Lashley jumping apparatus under increasing (3 mg/kg per week) doses of CDP. In 22 weeks of testing the doses increased from 3 to 65 mg/kg. The effect of the drug was to significantly lower response latency, reaching a maximum effect at 33 mg/kg. Then with higher doses the effect declined and at 48 mg/kg the effect was lost. At increasingly higher doses latency markedly increased and the animals became so ataxic many of them could no longer respond. These results demonstrated that with continuous drug administration a vigorous motor task could occur under high doses of CDP (up to 45 mg/kg), but that ultimately with still higher doses the compensations of tolerance failed.

Finally, the data in the present study show that no dose of CDP suppressed muricide in all rats on the first day of testing. Failure to suppress muricide might have been the result of inadequate dosing, administration of the drug into abdominal tissue or fat leading to slow absorption, or the presence of rats in the experiment that were marginally sensitive to the effects of CDP.

For the 75 mg/kg group, of the three rats that killed on the first drug day, one rat killed only once more on the third test. The other two rats killed at random on 4 more and 3 more test days respectively. It would appear that the first rat probably was not properly injected on two occasions. The seemingly random effectiveness of the drug for the other 2 rats suggest that they were only marginally responsive to the drug and killing was possibly determined by mouse related factors. Previously Van Hemel and Calucci [23] have shown that movement of the mouse is an important part of the stimulus complex that elicits attack. In the present study on more than one occasion the experimenter noticed a stand off between an ataxic rat and a mouse that bit back at the rat during attempted muricide. The rat then often retreated and crouched immobile for the remaining part of the 15 min test period while the mouse wandered around the cage unmolested.

For the 50 mg/kg group, 2 rats killed on every drug day, while 2 others showed random effectiveness of the drug suggesting marginal sensitivity.

Among the 25 mg/kg group, of the 6 killers on the first drug day 3 killed on every test, and 2 killed on every test but one. The sixth rat killed 3 more times at random. Here it seems there were 5 non-reactive rats but this would be expected as dose was reduced.

These data indicate that there is considerable within subject variance among muricidal rats with respect to the response to CDP. Further, it appears that mouse related factors may become significant when marginally reactive rats are challenged in a muricide test.

## EXPERIMENT 2

Assuming that drug tolerance was responsible for lower muricide latency over days in Experiment 1, a second experiment was done to establish what the mechanism of tolerance was. Drug tolerance in these experiments can be considered to be of two types, drug disposition tolerance which is caused by an increased rate of elimination or deactivation of the drug [13], or a behavioral tolerance which is an acquired accommodation to the drug and a return to normal functioning [3]. The possibility exists that the tolerance that was demonstrated in Experiment 1 could have been of the latter type and was merely a manifestation of the animals' acquired ability to kill mice in the presence of the drug.

To investigate that possibility, muricidal rats were given CDP daily for a number of days without muricide tests, then tested for muricide for one day under drug conditions. It was hypothesized on the one hand, that since these rats would have no opportunity to learn to kill mice under the influence of CDP, the absence of muricide on the drug-test day for this group would argue that a behavioral tolerance had occurred in Experiment 1. On the other hand, if the animals showed undiminished muricidal activity on the drug-test day it would argue for a drug disposition tolerance in both experiments.

*Method*

Twenty-eight Sprague-Dawley rats from 5–7 months old and weighing 375–540 g were used. Each rat was found to consistently kill when mice were placed in its home cage. On Days 1–3 each rat was given a saline injection (0.9%, 1 cc/kg) followed 30 min later with a muricide test as described in Experiment 1. On Days 4–10, each rat was given an IP injection of CDP or saline according to the following schedule. Six rats received 25 mg/kg, 9 rats received 50 mg/kg, 6 received 75 mg/kg, and 6 received saline. No muricide tests were given on these days. On Day 11, each rat was given the usual CDP dose followed 30 min later by a muricide test. The six rats that had received saline on Days 4–10 now received CDP, 50 mg/kg, followed by a muricide test. Latency of the kill was recorded. A maximum of 15 min was allowed for each test.

*Results and Discussion*

First, all rats killed mice during the first 3 saline tests. Second, all rats survived the drug injection regimen except for one rat in the 75 mg/kg group that died after 2 injections. With respect to muricide, the results were that all CDP treated rats killed mice during the muricide test under drug conditions (Day 11). Among the saline treated rats that received only one 50 mg/kg dose on the drug test day, 4 rats failed to kill during the 15 min test, and 2 killed after 70 and 90 sec respectively. Comparing the latency of the kill for the third no drug day (Day 3) and the drug test day (Day 11) (Wilcoxen matched-pairs signed-ranks test), showed that there was a significant increase in kill latency for the 25 and 75 mg/kg group ($p<0.05$) but there was no significant increase for the 50 mg/kg group. For the rats receiving saline on Day 4–10, the latency difference between Day 3 and Day 11 was also significant (non killers were assigned a latency of 15 min, $p<0.05$).

These results are consistent with the results of Experiment 1 in that continuous dosing with 50 mg/kg of CDP resulted in muricide latency under drug conditions not significantly different from control latency under saline, whereas dosing with 25 and 75 mg/kg led to significant increase in muricide latencies even though all rats killed mice within the 15 min test period. Further, the control animals that received saline for 7 days showed that an initial dose of 50 mg/kg is highly effective in supressing muricide and that the high levels of muricide on Day 11 for the 3 drug groups were not due to the absence of muricide tests for 7 days after the initial 3 day period of mouse killing. Another finding was that the 25 and 50 mg/kg of CDP daily seemed by subjective appraisal to recover from the effects of the drug in 24 hours, whereas the rats receiving 75 mg/kg seemed heavily sedated and delapidated when they were to be injected. This condition, however, was much reduced toward the end of the drug series. All of these data strongly indicate that tolerance to the depressant effect of the drug had developed during the daily administration of the drug. These observations are consistent with the findings of Hoogland *et al.* [9], who demonstrated the metabolic basis of tolerance, and Goldberg *et al.* [6] and Margules and Stein [16] who showed tolerance to the behavioral depressant effects of the benzodiazepines. Also, the high rate of mouse killing among these chronically dosed rats suggests that there is probably no specific anti-muricidal effect of CDP, and the suppression of this form of aggression in Experiment 1 was due to general behavioral depression. Furthermore, the fact that all the animals killed on their first test day under the drug condition eliminates the possibility that the rats in Experiment 1 had acquired a behavioral tolerance and merely needed practice under the drug state to again become consistent killers.

One finding that is not explained in Experiment 2 is the 100 percent rate of killing for the rats in the 3 drug groups on Day 11, while in Experiment 1 no such consistency occurred. It is true that these two experiments were run at different times partly by different experimenters, but there is no other known variable to account for these differences.

## EXPERIMENT 3

In order to further support the earlier finding that behavioral depression is the significant factor in reducing mouse-killing with CDP, a CNS stimulant was used to antagonize the behavioral depressant effect of CDP. In this case, caffeine was chosen since it has previously been shown to have effectively reversed CDP depression of the righting reflex [22] and antagonized CDP depression of exploratory behavior [18].

*Method*

Ten male Sprague-Dawley albino rats which were consistent mouse-killers were used. They were approximately 6 months old and weighed 400–500 g at the time of testing. They were housed separately and fed and watered ad lib. Each animal was tested a total of 6 days in the manner described in Experiment 1. However, saline (0.9%, 1 cc/kg IP) was administered 30 min before testing on Days 1 and 2, caffeine (75 mg/kg) on Days 3 and 4, and CDP (50 mg/kg) plus caffeine (75 mg/kg) on Days 5 and 6.

*Results and Discussion*

All animals killed mice within 15 min on each of the 6 tests whether they were administered saline or caffeine, or caffeine plus CDP. This result contrasts with the marked suppression of mouse-killing after initial tests with 50 mg/kg CDP in Experiment 1. However, a comparison of the latencies of mouse-killing showed that on Days 5 and 6 (CDP plus caffeine) even though all rats killed within the 15 min test, latencies were significantly longer than those on Days 1 and 2 (saline) ($t = 2.60$, $p<0.05$). But the latency for muricide under CDP and caffeine was still lower than that for the first 2 CDP tests for the 50 mg/kg group in Experiment 1, ($t = 6.88$, $p<0.01$). This shows that the concomitant administration of caffeine with CDP antagonized the anti-muricide effect, but in the doses used, the CDP effect was not completely reversed by caffeine. Our observations were that the CDP plus caffeine treated rats made numerous attempts to catch the mice but showed a degree of incoordination that often delayed the capture and kill. In contrast to the results found by Sofia [20] who found diminished rotorod activity and muricide with a 75 mg/kg dose of caffeine, muricide in our study was not affected by caffeine at that dose.

## GENERAL DISCUSSION

These experiments demonstrated that repeated administration of CDP resulted in a reduction of the anti-muricidal effect of the drug. These data were supported by the additional finding that chronic administration of CDP in the

absence of muricide tests also reduced the anti-muricide effect of the drug during a subsequent drug test session. This suggests that a drug disposition tolerance had probably occurred, a finding that is supported by the results of direct tests by Hoogland *et al.* [9]. Further support for these results were found when the anti-muricidal effect of CDP was to a significant extent reversed by the stimulant drug caffeine since the pharmacologic property of CDP which is subject to tolerance is a generalized behavioral depression.

In Experiment 1, a dose related suppression of muricide was not found. Nevertheless, there was evidence that there were differences between the 50 mg/kg dose group and the others. The evidence revealed a significant pattern of initial suppression followed by a return to muricide activity with continued dosing for this group. This suggested that this dose was the optimal dose for the detection of muricide suppression and tolerance to the effect.

In a subsequent paper it will be shown that there are dose related tolerance effects, but that the behavioral conditions under which tolerance is revealed are instrumental in determining tolerance dose parameters.

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