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Naloxone and Chlordiazepoxide: Effects on Acquisition and Performance of Signalled Punishment

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TRIPP, G. AND N. McNAUGHTON. *Naloxone and chlordiazepoxide: Effects on acquisition and performance of signalled punishment.* PHARMACOL BIOCHEM BEHAV **38**(1) 43–47, 1991.—The opiate antagonist, naloxone, has been reported to attenuate the loss of behavioural inhibition produced by benzodiazepines in tasks involving punishment and nonreward. This has led to suggestions that endogenous opioid systems may be involved in the anxiolytic actions of the benzodiazepines. However, the capacity of naloxone to block the effects of benzodiazepines depends on the behavioural schedule used. We tested the effects of naloxone and chlordiazepoxide on acquisition and performance of a signalled punishment schedule. Chlordiazepoxide (5 mg/kg IP) increased both punished and unpunished responding during acquisition and unpunished responding during performance of the conflict schedule. Naloxone (3 mg/kg IP) was essentially without effect on responding and failed to attenuate the punishment-releasing effects of chlordiazepoxide. The failure of naloxone and chlordiazepoxide to interact during acquisition of this punishment schedule is similar to results we obtained with successive discrimination and is in contrast to our findings with a differential reinforcement of low rates schedule. These results are consistent with the view that benzodiazepines reduce behavioural inhibition through two separate routes; that one of these routes depends on the release of endogenous opiates; and that the predominant route depends on schedule parameters.

Naloxone Chlordiazepoxide Signalled punishment Anxiolytics Benzodiazepines Rat

OPERANT conflict paradigms have been used extensively in the preclinical assessment of anxiolytic drugs. Clinically effective antianxiety agents, such as the benzodiazepines, typically restore responding suppressed by such schedules. These tasks have also been used to determine the involvement of endogenous opioid systems in the anxiolytic actions of the benzodiazepines.

The opiate antagonist naloxone has been reported to block the release of punished responding, observed with both diazepam and chlordiazepoxide (1, 6, 9, 12). However, the use of painful electric shock in these test schedules led to suggestions that naloxone was altering the animal's sensitivity to pain, not its response to the conflict situation (12). More recently naloxone has been shown to block the increase in burst responding and the shift of the interresponse time distribution curve accompanying chlordiazepoxide administration during acquisition of differential reinforcement of low rates of response (DRL) (15). This finding demonstrates that naloxone's ability to attenuate the anticonflict effects of chlordiazepoxide is not dependent on the inclusion of a nociceptive component, thereby legitimizing the use of punishment schedules in assessing endogenous opioid involvement in the anxiolytic actions of the benzodiazepines.

Published reports on the ability of naloxone to attenuate the anticonflict effects of the benzodiazepines on punishment schedules are, however, contradictory. Within the same article Soubrie et al. (12) report that a) naloxone blocked the increase in punished responding observed with diazepam during acquisition of an unsignalled conflict procedure; and b) it failed to attenuate the benzodiazepine-induced increase in responding during performance of a task involving a signal previously paired with electric shock. Obviously naloxone's ability to block the anticonflict effects of the benzodiazepines is not universal. Altering the experimental task parameters can interfere with the naloxone-benzodiazepine interaction.

We previously hypothesized (15) that naloxone would only block the effects of the benzodiazepines during acquisition of unsignalled conflict tasks. This suggestion is consistent with naloxone's reported ability to attenuate the anticonflict effects of chlordiazepoxide during acquisition but not performance of DRL (15) and its failure to interact with this benzodiazepine during either acquisition or performance of a signalled successive discrimination schedule (14). It also acknowledges McNaughton's (10) finding that chlordiazepoxide affects acquisition and performance through different mechanisms: a 'truly anxiolytic' action during acquisition and a 'state-dependent' action during performance.

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This account of naloxone-benzodiazepine interactions is helpful in explaining some contradictory findings (12, 14, 15). However, the results of Koob et al. (9) suggest that neither the phase of training nor the presence of a signal of conflict is critical to a naloxone blockade of the anticonflict actions of the benzodiazepines. In their study, naloxone attenuated the anticonflict effects of chlordiazepoxide during well-learned performance of the signalled punishment, but not the signalled nonreward (time-out) component of a 3-component multiple schedule. The lack of interaction in the nonreward component is consistent with our previous results (14), but the interaction during *performance* of a *signalled* punishment schedule seems contrary to both our results (14,15) and those of Soubrie et al. (12).

While the experimental parameters critical to the naloxone-benzodiazepine interaction have yet to be isolated, it is clear that changes in the conflict schedule significantly affect whether or not such an interaction occurs. The present experiments were designed to test the effects of naloxone and chlordiazepoxide on acquisition and performance of a signalled punishment procedure. Comparing acquisition and performance was important because this variable determines the drug interaction with DRL schedules (15) and because Soubrie et al. (12) did not investigate acquisition of their signalled punishment schedule. The schedule chosen was similar to that used in our previous experiments with nonreward (14). This schedule matching was important because of the apparent relation of the interaction to the inhibitor in Koob et al.'s (9) study. It was hoped that the results from these experiments would clarify the conditions necessary for the naloxone-benzodiazepine interaction.

Two experiments were carried out, the first tested the effects of naloxone 3 mg/kg and chlordiazepoxide 5 mg/kg on acquisition of a signalled punishment task, the second tested the same doses of these drugs on well-learned performance of the same schedule.

METHOD

Subjects

Subjects for Experiment 1 were 40 naive male Sprague-Dawley rats, weighing between 350 and 450 g at the beginning of training. The eight control animals from this experiment served as subjects for Experiment 2. All animals were group housed in a temperature-regulated room (21 ± 2 degrees C) on a 12-hour light/dark cycle. They were placed on a 23-hour food deprivation schedule 10 days before training began and maintained on this throughout the experimental period. Water was freely available in the home cages.

Apparatus

Four Campden Instrument operant boxes (24.5 by 22.5 by 23 cm) with grid floors, connected to Campden Instrument shock sources and shock scramblers, were used to train and test the animals. Each box was fitted with a food hopper and one fixed and one retractable lever. Only presses on the retractable lever had programmed consequences. With the exception of magazine training the retractable lever was extended into the box throughout each session. Three (2.8-watt) stimulus lights mounted above the levers and food hopper were used as the visual signal for both experiments. The experiments were controlled and data were recorded by Acorn Atom microcomputers programmed in ONLI-BASIC.

Procedure

Pretraining. Following ten days of 23-hour food deprivation the rats were magazine trained using a noncontingent Random Time 30-second (RT30) schedule. The computer selected intervals between 0 and 60 seconds using a random number generator and at the end of the interval a 45 mg Noyes reward pellet was delivered. Each magazine training session lasted 15 minutes. After four days of training the RT30 schedule was discontinued and the retractable lever extended into the box. Food pellets were now available on a continuous reinforcement schedule contingent on lever pressing. Three daily 30-minute lever press training sessions were run after which the eight rats with the lowest response rates were excluded from the remainder of Experiment 1. The remaining rats were then placed on an RI60-second schedule, similar to the RT30 but with food contingent on lever pressing. These sessions lasted for one hour and the rats were given daily sessions for seven days, rested for seven days and then given a further three days training on the schedule.

Signalled punishment training and drug treatments. Following pretraining the rats were assigned to one of the following drug groups (eight rats per group): saline vehicle; naloxone HCl 3 mg/kg; chlordiazepoxide HCl 5 mg/kg; naloxone HCl 3 mg/kg plus chlordiazepoxide HCl 5 mg/kg. The rats were given a further training session on the RI60 schedule during which the drugs were introduced. Before this session and all subsequent sessions the rats received two injections as follows: thirty minutes before the start of each session the rats received their first IP injection of either saline (0.9%) or naloxone HCl. Fifteen minutes before the start of the session they received their second IP injection of either saline (0.9%) or chlordiazepoxide HCl. All injections were given in a volume of 1 ml/kg. For the first drug session of RI60 the drug doses were 1.5 mg/kg naloxone HCl and 2.5 mg/kg chlordiazepoxide HCl. Thereafter, doses were naloxone HCl 3 mg/kg and chlordiazepoxide HCl 5 mg/kg. Following the first day of drug administration a visual signal (the three stimulus lights) was superimposed on the RI60 schedule. The three lights came on for four three-minute periods (at 12-minute intervals) during the 60-minute session. On the first day of presentation the visual signal had no programmed consequences thereafter it signalled that reinforced responses were accompanied by a brief electric shock (0.3 seconds). On the first day of signalled punishment the shock level was set at 0.1 milliamps (Campden Instruments), this was increased to 0.15 milliamps for the remainder of the experiment.

Experiment 2. At the end of Experiment 1, the eight saline control rats were randomly assigned to four groups of two and tested for a further eight days. Each group received each of the four drug combinations twice. The drug combinations were given in four different orders. If C = chlordiazepoxide HCl; N = naloxone HCl and S = saline, the orders were:

1. SS SC NS NC
2. NC SS SC NS
3. NS NC SS SC
4. SC NS NC SS

At the end of the first four days these orders were repeated.

Data Processing

For both experiments the computer cumulated separately the number of lever presses in the three-minute periods prior to (Pre-CS), and during (CS), operation of the punishment contingency. These cumulated response rates were printed out at the end of each 60-minute session and constituted the raw data for analysis. The data from Experiments 1 and 2 were logarithmically transformed to achieve normality of distribution and submitted to a) analysis of variance with comparison between Pre-CS and CS

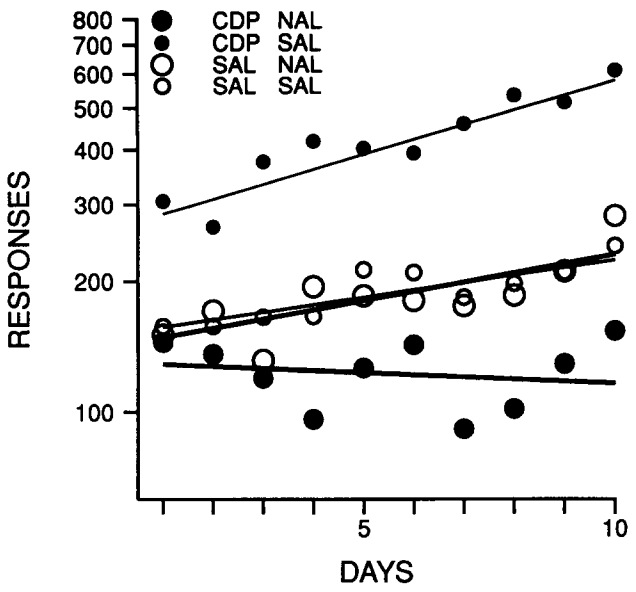

FIG. 1. Effects of chlordiazepoxide (CDP 5 mg/kg, IP) and naloxone (NAL 3 mg/kg, IP) given in combination with each other or with saline (SAL) on Pre-CS (rewarded) responding during acquisition of signalled punishment. The nonlinear response axis is the result of a logarithmic transform. The solid lines represent the linear regression lines for the individual groups.

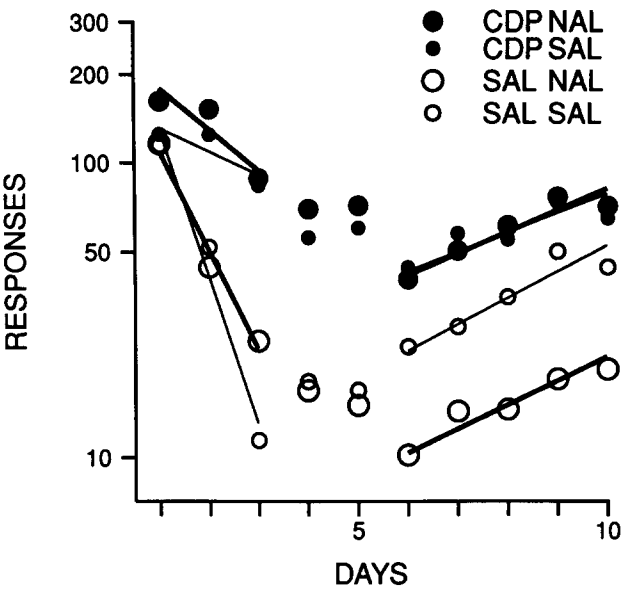

FIG. 2. Effects of chlordiazepoxide (CDP 5 mg/kg, IP) and naloxone (NAL 3 mg/kg, IP) given in combination with each other or with saline (SAL) on CS (punished) responding during acquisition of signalled punishment. The nonlinear response axis is the result of a logarithmic transform. Response rates have been adjusted for the Pre-CS response rate through analysis of covariance. The solid lines represent the linear regression lines, for the four groups, for days 1–3 and days 6–10, calculated from the post hoc analyses of variance carried out on these separate data sets.

generating a factor of 'discrimination' and b) analysis of covariance in which Pre-CS data were used as a covariate for CS data. All effects involving days were also assessed for the presence of consistent trends by extraction of orthogonal linear, quadratic, cubic and quartic components (11).

RESULTS

Experiment 1

The four drug groups showed steady development of discrimination over time [days × discrimination, linear component, $F(2,552) = 16.1$, $p<0.0005$] with considerable nonlinearity [quadratic, $F(2,552) = 33.5$, $p<0.0005$]. As can be seen in Fig. 1 chlordiazepoxide increased Pre-CS (unpunished responding) response rates in the saline control group, while those animals receiving both chlordiazepoxide and naloxone showed a decrease in responding. These opposing actions of chlordiazepoxide canceled one another statistically such that there was no evidence of a significant chlordiazepoxide main effect or days by chlordiazepoxide interaction. Naloxone alone did not affect responding, but interacted with chlordiazepoxide to decrease response rates in those animals receiving both drugs [linear component of the days × chlordiazepoxide × naloxone interaction, $F(1,276) = 15.4$, $p<0.0005$].

The CS (punished responding) response rates were adjusted for Pre-CS rates through analysis of covariance (see Fig. 2). Analysis of data from days 1–10 showed a significant days by chlordiazepoxide by naloxone interaction. However, it is clear from Fig. 2 that responding in the controls was biphasic with an initial rapid decrease in responding followed by a recovery phase. In addition, the saline animals began to recover after two days of signalled shock, the other groups did not show recovery of responding until the fifth day of shock.

Separate post hoc analyses of variance were, therefore, carried out on the data for days 1–3 and days 6–10. Linear regression lines from these separate analyses are plotted on Fig. 2. Over days 1–3 chlordiazepoxide, in combination with naloxone or saline, increased responding over that observed in rats treated with naloxone or saline alone. The magnitude of this effect increased across days [linear component of the days × chlordiazepoxide interaction, $F(1,55) = 22.2$, $p<0.0005$]. There was no evidence of a significant naloxone effect, and while naloxone and chlordiazepoxide appeared to interact, the effect did not reach an acceptable level of significance [linear component of the days × chlordiazepoxide × naloxone interaction, $F(1,55) = 2.9$, $0.05 < p < 0.1$].

As with days 1–3, over days 6–10 chlordiazepoxide increased punished responding in both the saline and naloxone animals [chlordiazepoxide main effect, $F(1,27) = 6.03$, $p<0.025$]. While naloxone appeared to reduce punished responding in the saline animals the effect was not statistically significant. There was no evidence of a significant naloxone by chlordiazepoxide interaction during this latter phase of acquisition.

Experiment 2

The results from performance of signalled punishment are presented in Table 1. There was clear evidence of discrimination across the four drug conditions, $F(1,32) = 179.6$, $p<0.0005$. While discrimination interacted significantly with both order of drug treatment, $F(1,32) = 16.9$, $p<0.0005$, and replication, $F(1,32) = 7.0$, $p<0.0005$, these factors did not interact significantly with naloxone or chlordiazepoxide. The eight experimental days were, therefore, pooled together and submitted to analysis of variance and analysis of covariance.

TABLE 1

EFFECTS OF CHLORDIAZEPOXIDE (CDP 5 mg/kg, IP) AND NALOXONE (NAL 3 mg/kg, IP) GIVEN IN COMBINATION WITH EACH OTHER OR WITH SALINE (SAL) ON PERFORMANCE OF SIGNALLED PUNISHMENT

	CDP NAL	CDP SAL	SAL NAL	SAL SAL
Pre-CS	2.477 (299.9)	2.501 (316.9)	2.328 (212.8)	2.324 (210.9)
CS	1.943 (87.7)	2.041 (109.9)	1.669 (46.7)	1.775 (59.6)
Difference	0.534 (N/A)	0.460 (N/A)	0.659 (N/A)	0.549 (N/A)
Cova CS	1.875 (74.9)	1.949 (88.9)	1.747 (55.9)	1.849 (70.6)

Response rates were measured preceding (Pre-CS) and during (CS) a stimulus signalling that reinforced responses would be accompanied by a brief electric shock on an RI60 baseline. The means in the main body of the table are the result of logarithmic transform of the raw data. Response rates given in parentheses are the result of inverse transform of the means not the means of raw data. The difference between Pre-Cs and CS rates is given as a measure of the discrimination within each group (inverse transform of these difference values is not meaningful) as are the adjusted group CS means from an analysis of covariance Cova (CS) using Pre-CS rates as covariate.

Chlordiazepoxide increased Pre-CS responding in both saline and naloxone animals [chlordiazepoxide main effect, $F(1,44) = 31.4$, $p<0.0005$]. Naloxone had no effect on the Pre-CS response rate (all F ratios associated with this factor were less than 2.0). Covariance analysis of the CS data showed that while chlordiazepoxide appeared to increase responding in both saline and naloxone animals this effect was not statistically significant. Although naloxone appeared to decrease responding in both the saline and chlordiazepoxide groups the effect was not significant. There was no evidence of a significant naloxone-chlordiazepoxide interaction and naloxone did not reduce the effect of chlordiazepoxide.

DISCUSSION

The results from acquisition of signalled punishment, showing a chlordiazepoxide-induced increase in both punished and unpunished responding, are consistent with reports that the benzodiazepines enhance consummatory activities (4) and increase rates of punished responding (3,5). Naloxone alone had no effect on unpunished responding, and did not significantly alter rates of punished responding in the early (days 1–3) or later stages (days 6–10) of acquisition. Administered together with chlordiazepoxide, naloxone failed to block the punishment-releasing effects of chlordiazepoxide during either stage of acquisition. It did, however, block the increase in unpunished responding produced by chlordiazepoxide. This finding, while consistent with reports that naloxone reverses benzodiazepine-induced increases in food consumption (2,13), differs from the results obtained with successive discrimination (14). Under the successive discrimination schedule chlordiazepoxide alone had no effect on food maintained responding, whereas together with naloxone it increased responding. Gray (7) suggests that the benzodiazepines do not show consistent effects on rewarded behaviour, while Iversen and Iversen (8) report that the benzodiazepines increase low rates of responding whether they are associated with punishment or reward. A comparison of Pre-CS (rewarded responding) response rates under successive discrimination (14) and the present signalled punishment schedule indicate a lower rate of responding with signalled punishment. This response rate difference may account for the differential effects of chlordiazepoxide on rewarded responding. Irrespective of the direction of the chlordiazepoxide effect, there was a significant naloxone-chlordiazepoxide interaction.

The failure of naloxone to attenuate the anticonflict effects of chlordiazepoxide during acquisition of signalled punishment, while not unexpected, is at variance with the findings of Soubrie et al. (12) and Duka et al. (6). However, neither of these schedules incorporated an explicit signal of changed reinforcement contingencies. In our previous experiments with nonreward, we found no evidence of a naloxone/benzodiazepine interaction in a signalled conflict task (14), but found the two drugs did interact during acquisition of unsignalled conflict (15).

When naloxone and chlordiazepoxide were tested during well learned performance of signalled punishment the results were essentially the same. Chlordiazepoxide appeared to increase both punished and unpunished responding (albeit nonsignificantly) and there was no sign of a naloxone-chlordiazepoxide interaction. In light of naloxone's failure to interact with chlordiazepoxide during acquisition of signalled punishment, the results obtained during performance of this schedule are not surprising. This result, while consistent with the findings of Soubrie et al. (12), contrasts markedly with the results of Koob et al. (9) who found naloxone attenuated the anticonflict effects of chlordiazepoxide during well-learned performance of signalled punishment.

The precise parametric basis for the discrepent results of Koob et al. (9) is not clear. The most obvious difference, the use of a 3-component schedule, seems intuitively unlikely. Some possible complications are that their saline animals show virtually total suppression in the punishment condition and relatively high rates of responding (90/minute) in the baseline condition which could have produced 'floor' and 'ceiling' contamination of the estimates of the drug interaction. The apparent naloxone attenuation of the anticonflict effects of chlordiazepoxide may reflect the combined independent actions of naloxone and chlordiazepoxide. Interestingly, naloxone and chlordiazepoxide were not found to interact during the successive discrimination (time out) phase of Koob et al.'s (9) three-component schedule.

The present results, with signalled punishment, are very similar to those obtained when nonreward was used to suppress responding instead of punishment (14). In both cases naloxone and chlordiazepoxide failed to interact in relation to behavioural inhibition during either the acquisition or performance phases of testing. Together with the clear interactions obtained with unsignalled punishment (6,12) and differential reinforcement of low rates of response (15), these results support the proposal that "transmission by opiate peptides may be involved in only some 'disinhibi-

tory' effects of benzodiazepines'' (12). They also suggest the nature of 'anxiety' generated by the various conflict schedules is different. Similar types of anxiety appear to be generated by classical successive discrimination and signalled punishment schedules which differ from that generated by DRL. While the animal's outward expression of 'anxiety' appears constant across all schedules (suppression of responding) the introduction of naloxone and chlordiazepoxide to the animal's system suggests that the processes responsible for maintaining these responses may differ depending on the schedule used.

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