*Pharmacology Biochemistry and Behavior*, Vol. 41, pp. 475–481, 1992
Printed in the U.S.A. 

0091-3057/92 $5.00 + .00


# Schedule Dependence of the Interaction of Naloxone and Chlordiazepoxide

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Received 17 April 1991

TRIPP, G. AND N. McNAUGHTON. *Schedule dependence of the interaction of naloxone and chlordiazepoxide.* PHARMACOL BIOCHEM BEHAV **41**(3) 475–481, 1992.—Reports that the opiate antagonist, naloxone, blocks the anticonflict effects of diazepam and chlordiazepoxide suggest endogenous opioid involvement in the anxiolytic actions of the benzodiazepines. However, naloxone's ability to antagonize the anticonflict effects of the benzodiazepines is not universal, but schedule specific. The present experiments investigated the importance of the timing of conflict periods and control of reinforcement on the naloxone–benzodiazepine interaction. We tested the effects of naloxone (3 mg/kg, IP) and chlordiazepoxide (5 mg/kg, IP) on acquisition of a successive discrimination schedule, with nonreward periods similar in length and frequency to those of signalled DRL, and on an FI60-s schedule. Chlordiazepoxide increased rewarded responding and, unexpectedly, decreased nonrewarded responding during acquisition of successive discrimination. This reduction in nonrewarded responding was reversed by naloxone. Under the FI60 schedule, chlordiazepoxide increased nonrewarded responding, an effect that was totally blocked by naloxone at the beginning of the FI. Naloxone's ability to reverse the response-releasing effect of chlordiazepoxide decreased later in the FI. These results suggest endogenous opioid systems are involved in the anxiolytic actions of the benzodiazepines when the animal is adapting to recently introduced conflict. Once adaptation occurs, other neurotransmitter systems mediate the actions of the benzodiazepines.

Naloxone Chlordiazepoxide Successive discrimination FI60 Anxiolytics Benzodiazepines Rat

THE clinical anxiolytic actions of the benzodiazepines correlate highly with their ability to restore responding suppressed by punishment. Reports that the opiate antagonist, naloxone, blocked the anticonflict actions of diazepam and chlordiazepoxide (1,7,13,19) suggested the endogenous opioid systems might be involved in the anxiolytic actions of the benzodiazepines. Such a proposal is consistent with reports that benzodiazepine enhancement of GABAergic function underlines the anticonvulsant effects of these drugs (11), but does not account for all their anxiolytic actions (16).

Naloxone does not, however, universally block the anticonflict effects of diazepam (19) or chlordiazepoxide (20,21, 23). Soubrie et al. (19) reported that naloxone blocked the punishment-releasing effects of diazepam during acquisition of a conflict procedure but failed to reverse diazepam-induced increases in responding in the presence of a signal *previously* paired with electric shock. From this, they inferred a nociceptive component was essential to the naloxone–benzodiazepine interaction. The results of a study generating conflict through nonreward indicated this was not the case. Tripp et al. (23) found naloxone successfully blocked the response-releasing effects of chlordiazepoxide during acquisition of a differential reinforcement of low rates of response (DRL) procedure. Naloxone and chlordiazepoxide did not interact during well-learned performance of this same task. This finding is consistent with McNaughton's (14) suggestion that chlordiazepoxide affects acquisition and performance of conflict schedules through different mechanisms: a "truely anxiolytic" action during acquisition and a "state-dependent" action during well-learned performance. Similar logic could be applied to Soubrie et al.'s (19) report that naloxone failed to interact with diazepam in the presence of a signal previously paired with electric shock—a performance-based schedule.

In subsequent experiments, we tested the effects of naloxone and chlordiazepoxide on acquisition and performance of successive discrimination (20) and signalled punishment (21). Not unexpectedly, naloxone failed to interact with chlordiazepoxide during well-learned performance of either schedule. However, naloxone did not interact with chlordiazepoxide during acquisition of either successive discrimination or signalled punishment.

An obvious procedural difference between DRL and both successive discrimination and signalled punishment schedules is the absence, in DRL, of a visual signal of conflict. The influence of an explicit signal of conflict on the naloxone–chlordiazepoxide interaction was examined in direct compari-

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son of the effects of naloxone and chlordiazepoxide on DRL and on signalled DRL (22). This latter schedule was identical to DRL except periods of nonreward were signalled visually. While the introduction of a visual signal of nonreward significantly altered the distribution of responses, naloxone continued to block the anticonflict effects of chlordiazepoxide. These results suggest that inclusion of a visual signal of conflict does not interfere with the naloxone–benzodiazepine interaction.

In addition to the lack of a visual signal of conflict, DRL differs from conventional successive discrimination and signalled punishment with respect to the timing of conflict periods and the ability of the subject to influence reinforcement opportunities. Under a DRL schedule, periods of conflict (nonreward) occur frequently and are of short duration. Furthermore, the rat's pattern of responding mediates the timing of reinforcement opportunities (i.e., if the rat responds before the conflict interval finishes the clock is reset and the interval is extended). Conversely, with both successive discrimination and signalled punishment, conflict periods occur infrequently, are of longer duration, and reinforcement availability is not affected by the animal's responses. These schedule differences may determine whether or not there is endogenous opioid involvement in anticonflict actions of chlordiazepoxide and therefore whether or not naloxone and chlordiazepoxide will interact under a conflict schedule.

The present experiments investigated the importance of the timing of conflict periods and the control of reinforcement on the naloxone–chlordiazepoxide interaction. Experiment 1 tested the effects of naloxone and chlordiazepoxide on acquisition of a successive discrimination schedule in which the frequency and duration of nonreward periods were like those of signalled DRL. However, unlike signalled DRL, the rat's responses did not alter the availability of reinforcement. In Experiment 2, we tested naloxone and chlordiazepoxide on an FI60-s schedule. Under an FI schedule, reinforcement becomes available at regular temporal intervals, that is, every 60 s, provided the rat makes an appropriate response. Like ordinary DRL, an FI60 schedule does not contain an explicit signal of nonreward. It differs from DRL in that the animal's responses do not affect reinforcement opportunities, and it differs from the present successive discrimination schedule (Experiment 1) in having somewhat longer nonreward intervals. With this schedule, we assessed the relationship of the drug effects to the time within the FI.

## METHOD

### *Subjects*

Subjects for Experiment 1 were 34 naive, male Sprague-Dawley rats, weighing between 350–600 g at the beginning of training. Subjects for Experiment 2 were a group of 40 naive, male Sprague-Dawley rats weighing between 350–450 g. All animals were group housed in a temperature-controlled room (21 ± 2°C) on a 12L:12D cycle with lights on at 6 a.m. At least 10 days before the beginning of training, rats from both experiments were placed on a 23-h food deprivation schedule that was maintained throughout the experimental period. Water was freely available in the home cages.

### *Apparatus*

Eight Campden Instrument operant boxes (24.5 × 22.5 × 23 cm) with grid floors were used to train and test the animals from Experiment 1. Fourteen similar boxes were used in Experiment 2. Each box contained a food hopper, one fixed lever and one retractable lever, three small 2.8-W lights positioned above the two levers and the food hopper, and a 2.8-W houselight in the center of the roof. Only presses on the retractable lever had programmed consequences for the present experiments. With the exception of magazine training, the retractable lever was extended into the operant box throughout each session. The three lights were used together as the discriminative stimuli for nonreward in Experiment 1. Experiment 1 was controlled, and data collected, by Acorn Atom microcomputers programmed in ONLIBASIC. A BBC microcomputer was used to control and collect data in Experiment 2.

### *Procedure*

*Pretraining (Experiments 1 and 2).* After at least 10 days of 23-h food deprivation, rats from both experiments were magazine trained using a noncontingent random time (RT 30) schedule. Under this schedule, the computer selected intervals between 0 and 60 s using a random number generator. At the end of each interval, a 45-mg (NOYES) reward pellet was delivered. The animals in Experiment 1 received one 15-min session per day for 3 days. In Experiment 2, subjects received a single session that lasted for 30 min.

Following magazine training, the RT 30 schedule was discontinued and food pellets were made available contingent on lever pressing. Each continuous reinforcement session lasted for 30 min and subjects in both experiments were given one session per day for 3 days. For the first of these sessions, wet mash was smeared on the retractable lever to encourage approach and manipulation.

### *Drug Treatments*

Following the third continuous reinforcement session, rats with the lowest response rates were dropped from Experiment 1 (two rats) and Experiment 2 (eight rats). Remaining rats, in each experiment, were then assigned to the following drug groups (eight rats per group): 0.9% saline vehicle; naloxone HCl 3 mg/kg; chlordiazepoxide HCl 5 mg/kg; and naloxone HCl 3 mg/kg plus chlordiazepoxide HCl 5 mg/kg. They were then run for an additional 2 (Experiment 1) or 3 days (Experiment 2) on the continuous reinforcement schedule. Before the first of these sessions, and all subsequent sessions, rats received two injections as follows. Thirty minutes before the start of each session, rats received their first IP injection of either saline 1 ml/kg or a 1-ml/kg solution containing naloxone HCl. Fifteen minutes before the start of the session, they received their second IP injection of either saline 1 ml/kg or a 1-ml/kg solution containing chlordiazepoxide HCl. For the first additional day of continuous reinforcement, 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. These doses were chosen to match those used in previous experiments (20–23).

### *Experiment 1—Successive Discrimination*

Following the additional 2 days of continuous reinforcement, rats were placed on a successive discrimination schedule designed to have the same distribution of reinforced and nonreinforced periods and stimuli as signalled DRL 15 s but without any response contingency. Under this schedule, the three

stimulus lights came on for an average of 20 s (15-s fixed interval plus RT5, which generated a random light interval of between 15 and 25 s with a mean interval length of 20 s) and went off again for an average of 5 s (RT5, which generated a random dark interval of between 0 and 10 s with a mean interval length of 5 s). These average interval lengths were calculated from the total light (nonrewarded) and dark (rewarded) time (seconds) divided by the number of light and dark intervals recorded under signalled DRL 15 (22). No reinforcements were available when the lights were on, although the rats were able to press the retractable lever. During the dark (reinforcement) phase, a single reinforcement was set up at least 1 s after stimulus lights went out. If the rat pressed the lever after this time had elapsed, it received a reward pellet. The 1-s delay in reinforcement availability was programmed to ensure the rat associated the lights-off condition with reinforcement. Only one reinforcement opportunity per lights-out period was permitted as under signalled DRL any lever-press response (including rewarded responses) reset the DRL interval; thus, during any dark phase the rat is only able to obtain one food reinforcement.

Due to an intermittent fault in the computer programming language, testing on this final schedule did not begin until 5 days after the end of continuous reinforcement. The rats were run on the faulty successive discrimination schedule for 2 days and then rested for 3 days while the fault was traced and corrected. The fault was that whenever an RT interval of 0 s was selected (on average once in $<500$ intervals, that is, once every 4.2 h per rat) the computer stopped the program, resulting in experimental extinction. One rat was dropped from the experiment as its lever-press response had extinguished by this time. The remaining rats were run on the corrected successive discrimination schedule for 16 days. The sessions lasted for 1 h and each rat was run in the same operant box at the same time each day. All rats were fed shortly after the last session of day.

### *Experiment 2—FI60*

At the end of continuous reinforcement, the 32 rats in Experiment 2 were placed on a FI60-s schedule. Under this schedule, the first lever-press response occurring after the passage of 60 s was rewarded. Rats were able to press the retractable lever throughout each 60-s interval; however, only the first response after the end of the interval was rewarded. The sessions lasted for 30 min and each rat received one session per day for 28 days. As for Experiment 1, each rat was run in the same operant box at the same time each day.

### *Data Collection and Analysis*

*Experiment 1.* The computer recorded the total number of response made in both the light (nonrewarded) and dark (rewarded) phases of the schedule. It also recorded the total time spent in each of these phases, which was different for the two phases and varied to some extent from session to session. At the end of each 60-min session, it printed out the response rate (per second) for both the light and dark phases of the schedule. These response rates constituted the raw data for analysis. The data was logarithmically transformed [$X' = \log 10\ (X + 1.0)$] to achieve normality of distribution (24) and then submitted to 1) analysis of variance (ANOVA) with between PreCS and CS comparison, generating a factor of "discrimination," and 2) analysis of covariance in which PreCS data were used as a covariate for CS data. For clarity of graphical representation, the data for the covariance analysis was analysed by pairs of days. All effects involving drugs and days were assessed for the presence of linear, quadratic, and cubic polynomial components (18). The linear component extracted by this method is identical to the slope of the least-squares linear regression applied to the same data. The higher-order components are symmetrical curves with increasing numbers of inflections.

*Experiment 2.* In Experiment 2, the computer recorded the total number of responses made by the rat and the timing of each response since the beginning of the 60-s interval in which it occurred. Responses were then binned according to when in the interval they occurred. Bin 1 received responses that occurred between 0 and 5 s after the interval began, bin 2 received responses that occurred between 6 and 10 s, and so on up until bin 12, which received responses made between 56 and 60 s after the interval began. The raw data underwent a square root transform [$X' = \mathrm{SQRT}(X + 0.5)$] to achieve normality of distribution and were submitted to ANOVA. All effects involving drugs, days, and bins were assessed for the presence of linear, quadratic, and cubic polynomial components. As in Experiment 1, the data was analysed as pairs of days.

## RESULTS

### *Experiment 1*

The four drug groups showed a steady development of discrimination across days [linear component of the days × discrimination interaction, $F(1,492) = 274.4$, $p < 0.0005$], with some nonlinearity [quadratic component, $F(1,492) = 18.6$, $p < 0.0005$]. Those animals receiving CDP alone showed better discrimination than the saline control animals.

When the CS response rate was adjusted for the PreCS response rate through analysis of covariance (see Fig. 1), those animals receiving chlordiazepoxide showed a decrease in nonrewarded responding relative to the non-chlordiazepoxide animals [linear component of the daypair × chlordiazepoxide interaction, $F(1,432) = 28.1$, $p < 0.0005$]. This effect was greatest in animals receiving chlordiazepoxide and saline. Naloxone interacted significantly with chlordiazepoxide to attenuate its decrease in nonrewarded responding [linear component of the daypair × chlordiazepoxide × naloxone interaction, $F(1,432) = 17.8$, $p < 0.0005$].

PreCS response rates for the four experimental groups are shown in Fig. 1. Chlordiazepoxide increased PreCS responding in both the saline and naloxone groups [chlordiazepoxide main effect, $F(1,27) = 9.9$, $p < 0.005$]. Naloxone significantly decreased PreCS responding [naloxone main effect, $F(1,27) = 4.4$, $p < 0.05$]; its effect was greatest in saline animals. The effect of chlordiazepoxide was greatest in the naloxone animals [linear component of the daypair × chlordiazepoxide × naloxone interaction, $F(1,432) = 5.5$, $p < 0.05$] and it blocked naloxone's decrease in PreCS responding.

### *Experiment 2*

Under the FI60 schedule, chlordiazepoxide increased nonrewarded responding in both naloxone and saline animals [chlordiazepoxide main effect, $F(1,31) = 11.2$, $p < 0.0025$]. As can be seen from Fig. 2, the extent to which chlordiazepoxide released responding varied across bins [linear, quadratic, and cubic components of the bins × chlordiazepoxide interaction, lin $F(1,9559) = 214.8$, $p < 0.0001$; quad $F(1,9559)$

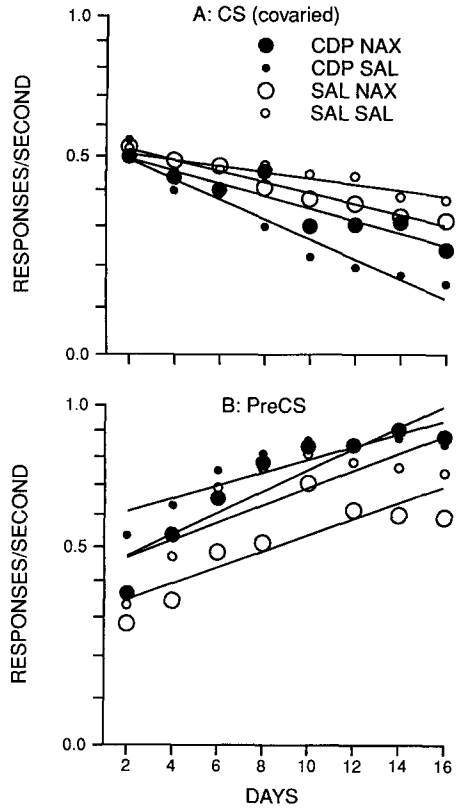


FIG. 1. Effects of chlordiazepoxide (CDP, 5 mg/kg IP) and naloxone (NAX, 3 mg/kg, IP) given in combination with each other or with saline (SAL) on responding during acquisition of a successive discrimination schedule designed to model DRL. The nonlinear response axis is the result of a logarithmic transform. Solid lines represent the linear regression lines for the four groups. CS (nonrewarded) response rate, adjusted for PreCS rate by analysis of covariance: Chlordiazepoxide decreased nonrewarded responding, an effect blocked by naloxone. PreCS (rewarded) responding: Chlordiazepoxide increased rewarded responding in both the saline and naloxone animals, while naloxone decreased such responding. This naloxone effect was blocked by chlordiazepoxide.

= 381.0, $p < 0.0001$; cub $F(1,9559) = 31.2$, $p < 0.0001$]. This response-releasing effect of chlordiazepoxide was totally blocked by naloxone at the beginning of the FI interval and the blocking effect progressively decreased so that from about 30–60 s it was negligible or absent [bins × naloxone × chlordiazepoxide, linear $F(1,9559) = 112.2$, $p < 0.0001$; quad $F(1,9559) = 10.7$, $p < 0.0025$; cub $F(1,9559) = 12.7$, $p < 0.0005$]. Figure 2 shows that for 0–5 s and 6–10 s the response rates of rats receiving both naloxone and chlordiazepoxide were closer to those of the saline control group than to either the naloxone or chlordiazepoxide groups alone. However, later in the interval the response rates for rats receiving both naloxone and chlordiazepoxide more closely approximated those of the chlordiazepoxide-only group. By 56–60 s, it is difficult to distinguish between the two chlordiazepoxide groups.

In addition to its interaction with the response-releasing effects of chlordiazepoxide, naloxone showed specific effects of its own. During the earlier bins, naloxone significantly reduced responding in the saline animals [linear, quadratic, and cubic components of the bins × naloxone interaction, lin $F(1,9559) = 316.7$, $p < 0.0001$; quad $F(1,9559) = 51.1$, $p < 0.0001$; cub $F(1,9559) = 85.2$, $p < 0.0001$], an effect that was not evident in later bins (see Fig. 2, 21–25 and 56–60 s).

The binned response rate data suggests that rats in all experimental groups learned the temporal discrimination. There was a decrease, across days, in responding in early bins (Fig. 2, 0–5 and 6–10 s), while responding in later bins, particularly those close to the interval length, increased (Fig. 2, 56–60 s). This effect is demonstrated statistically by highly significant bins × daypair interactions [lin·lin $F = 1000.7$; quad·lin $F = 44.4$; cub·lin $F = 109.3$; quad·quad $F = 66.8$; lin·quad $F(1,9559) = 34.5$, $p < 0.0001$].

## DISCUSSION

Results from previous experiments with naloxone and chlordiazepoxide (20–23) suggest that endogenous opioids are involved in the anxiolytic actions of the benzodiazepines during the acquisition of tasks in which periods of conflict occur frequently and are of short duration, that is, when the experimental animal is required to adapt to a rapidly changing environment. The two experiments described in this article were designed to extend our understanding of the conditions necessary for a naloxone–benzodiazepine interaction.

In Experiment 1 (successive discrimination), chlordiazepoxide increased rewarded (PreCS) responding, while it decreased nonrewarded (CS) responding. These results suggest chlordiazepoxide actually improved the rat's ability to discriminate between the rewarded and nonrewarded phases of the schedule. Naloxone alone decreased PreCS (rewarded) responding, an effect that was attenuated by chlordiazepoxide. While it had no effect on nonrewarded (CS) responding, naloxone interacted with chlordiazepoxide to reverse the benzodiazepine's decrease in such responding.

The opposing actions of naloxone and chlordiazepoxide on rewarded responding are consistent with reports that, while the benzodiazepines enhance consummatory activities (4), naloxone suppresses food intake in the rat (2,3,8,12,17). Most researchers report that naloxone blocks benzodiazepine enhancement of consummatory activities; however, the opposite happened in this experiment, with chlordiazepoxide attenuating naloxone's suppression of food-reinforced responding. While this result is not usual, Cooper (3) also reported that chlordiazepoxide blocked a naloxone-induced decrease in drinking.

Unlike its effect on rewarded (PreCS) responding, the actions of chlordiazepoxide on nonrewarded (CS) responding are completely unexpected. Chlordiazepoxide *decreased* the CS response rate, a result diametrically opposed to previous reports that the benzodiazepines impair successive discrimination by releasing nonrewarded responding [see (5,9,10,14) for reviews]. There are, however, reports in the literature of chlordiazepoxide failing to alter the rate of bar pressing in time-out (successive discrimination) schedules. Miczek (15) reported that, while chlordiazepoxide consistently enhanced behavior that was suppressed by various punishment procedures, response suppression due to nonreinforcement remained unaltered by chlordiazepoxide. Similarly, Dantzer and Baldwin (6) reported only mild disinhibition of nonreinforced responding in pigs. They suggested the absence of a significant chlordiazepoxide effect was due to either a difference in pig metabolism of the drug or some species-specific behavior that interfered with the operant response.

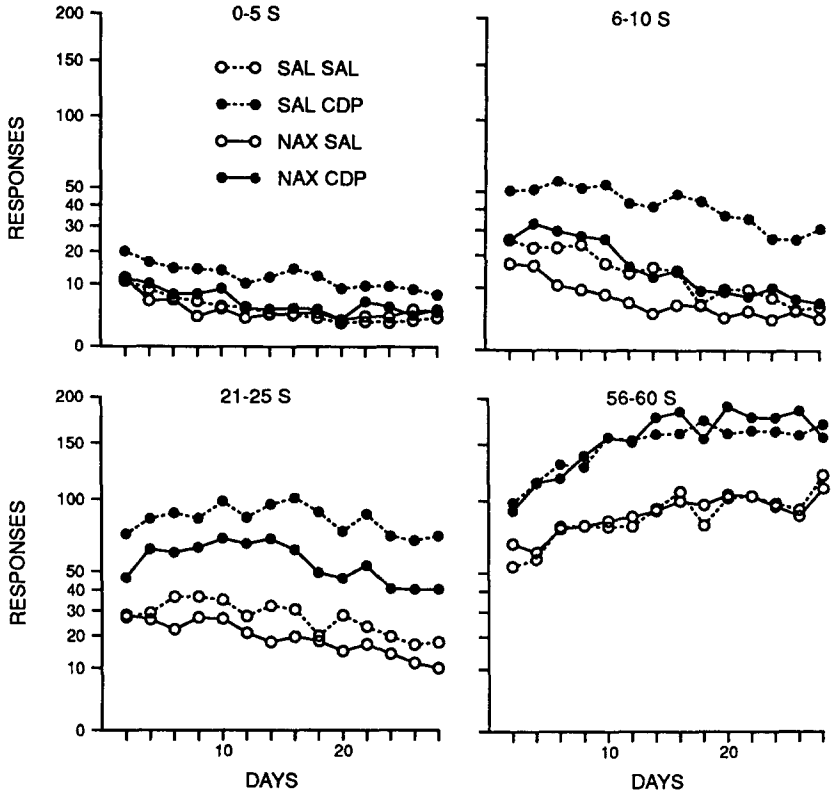


FIG. 2. Effects of chlordiazepoxide (CDP, 5 mg/kg, IP) and naloxone (NAX, 3 mg/kg, IP) given in combination with each other or with saline (SAL) on a FI 60-s task. The data is response rates per 5-s bin. Pairs of adjacent days have been pooled for clarity of graphical representation. The nonlinear response axis is the result of a square foot transform. The data from the four 5-s bins presented were chosen to illustrate the trend in the pattern of responding across all 12 5-s bins. 0–5 s: Chlordiazepoxide releases nonrewarded responding, an effect blocked by naloxone. Over the 28 days of the experiment, there is a general decrease in nonrewarded responding in this early bin. 6–10 s: Chlordiazepoxide continues to release nonrewarded responding. This effect, while more obvious than in the 0–5-s bin, is reduced by naloxone, especially later in acquisition. Naloxone shows response-inhibiting effects of its own. 21–25 s: Chlordiazepoxide increases responding in both the naloxone and salien animals more than in the previous two cases. Naloxone continues to inhibit responding but reduces the effects of chlordiazepoxide only marginally. 56–60s: Chlordiazepoxide releases responding equally in saline and naloxone animals. This effect is similar in size to that at 21–25 s. Naloxone no longer shows an response-inhibiting action of its own, nor does it interact with chlordiazepoxide.

It is possible that the present chlordiazepoxide-induced decrease in nonrewarded responding was due to chlordiazepoxide releasing other behaviors that compete with the lever-pressing response. If rearing, waiting, or exploratory behavior were released by chlordiazepoxide, this response would occur with greater frequency, leaving the rat less time for lever pressing. As the rats in this study were not observed for the duration of each experimental session, there is no direct evidence of what this behavior might have been or in fact that any other behavior was released. Whatever the cause of the chlordiazepoxide-induced reduction in nonrewarded (CS) responding, the effect was blocked by naloxone. This suggests that as with DRL and signalled DRL the effects of chlordiazepoxide on this conflict schedule may be endogenous opioid mediated.

It is clear from these results that altering the frequency and duration of nonreward periods substantially changes the nature of the successive discrimination task. It does not, however, produce a schedule equivalent to signalled DRL. The effects of chlordiazepoxide on acquisition of the present successive discrimination schedule are distinct from its effects on acquisition of signalled DRL (22). This suggests that the differences between signalled DRL and successive discrimination extend beyond timing of the presentation of nonreward.

As noted earlier, signalled DRL and successive discrimination also differ in the extent to which the experimental animal can influence the availability of reinforcement opportunities. Under a signalled DRL schedule, the animal has some control over the length of the nonrewarded (CS) phase and hence the frequency with which reinforcement becomes available. If the animal responds at the end of the nonreward interval, it receives a reward pellet and the duration of the nonreward phase remains close to the criterion interval length. However, if the animal responds within the interval no reinforcement is made available and the nonreward interval is reset, increasing the

duration of that nonreward period. With successive discrimination, the animal's pattern of responding has no effect on the length of the CS period or the availability of reinforcement. While having some control over reinforcement opportunities appears to alter the animals responding under naloxone and chlordiazepoxide, it does not prevent these drugs from interacting.

Regardless of the differences in responding reported under signalled DRL (22) and the present successive discrimination schedule, the effects of chlordiazepoxide under both were blocked by naloxone. This implies that in both schedules the effects of chlordiazepoxide were endogenous opioid mediated, a finding consistent with the hypothesis that the timing of the conflict periods rather than stimulus presentation or schedule control is a critical factor in determining whether naloxone will interact with chlordiazepoxide.

The FI60 schedule described in Experiment 2 allowed us to assess the effect of naloxone and chlordiazepoxide on nonrewarded responding at varying intervals postreward in the absence of either explicit signalling of the nonreward interval or schedule. Under this schedule, chlordiazepoxide increased nonrewarded responding in both saline and naloxone animals. This response-releasing effect of chlordiazepoxide was effectively blocked by naloxone in the early bins. With increasing bin length, the response rates of animals receiving both naloxone and chlordiazepoxide approached those of the chlordiazepoxide-only animals. In addition to blocking the response-releasing effects of chlordiazepoxide, naloxone alone decreased nonrewarded responding. This effect decreased with increasing bin number. Irrespective of the drugs they received, the four groups in this experiment learned the temporal contingencies surrounding reward. Across experimental days, there was a decrease in responding in early bins, while responding in later bins (particularly those close to the fixed-interval length) increased. It should be noted that the effect of naloxone on chlordiazepoxide-treated animals was greater than on saline-treated animals. The effects of naloxone both subtract from and genuinely block the effects of chlordiazepoxide on this task.

The results from Experiment 2 indicate that under the FI60 schedule the anticonflict effects of chlordiazepoxide are endogenous opioid mediated during the early, but not later, part of the nonreward interval. Until approximately 15 s into the FI60 interval, naloxone continues to block the response-releasing effects of chlordiazepoxide. Beyond this, those animals receiving naloxone and chlordiazepoxide exhibit response patterns increasingly similar to those of the chlordiazepoxide-only animals.

Thus while the timing of conflict periods appears important to the naloxone–benzodiazepine interaction it may not be the frequency or duration of such periods that is critical, but rather when in the conflict interval responding occurs. The anticonflict effects of chlordiazepoxide early in the interval appear opioid mediated, while such effects later in the interval would seem to involve another neurotransmitter system.

If correct, this explanation of naloxone–benzodiazepine interaction accounts for the variable effects of these drugs in all the conflict schedules tested. Under both DRL and signalled DRL, naloxone blocked the response-releasing action of chlordiazepoxide on premature responding; however, the effect of these drugs on burst (very early) responding was variable (22,23). The absence of a naloxone–benzodiazepine interaction during burst responding is not unexpected as such responding can occur anywhere in the nonreward interval. Naloxone and chlordiazepoxide also failed to interact during signalled punishment and successive discrimination (20,21). Response rates for both experiments were cumulated over each conflict period, not binned as in DRL or FI60. Thus, we cannot say with certainty that naloxone failed to block the anticonflict actions of chlordiazepoxide immediately following the presentation of conflict in these schedules.

It would seem then that endogenous opioid systems are involved in the anxiolytic actions of the benzodiazepines when the animal is adapting to recently introduced conflict (nonreward in the present experiments). The mechanism involved is unclear, but it is more likely that the benzodiazepines act to release an endogenous opioid than that they act directly at opioid receptors. Once adaptation has occurred, that is, later in the conflict interval or during well-learned performance, the benzodiazepines exert their anxiolytic effects through mechanisms other than those involving the endogenous opioid system.

ACKNOWLEDGEMENTS

This research was supported by grant 86/104 of the Medical Research Council of New Zealand (MRCNZ). N. M. was an MRCNZ Senior Fellow. G. T. received a University Grants Committee of New Zealand postgraduate research scholarship. Chlordiazepoxide was donated by F. Hoffmann-La Roche & Co. and naloxone by Du Pont.

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