The role of brain amines in the conditioned avoidance response; chlordiazepoxide facilitation of 2-way avoidance.

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THE ROLE OF BRAIN AMINES IN THE CONDITIONED AVOIDANCE RESPONSE: CHLORDIAZEPoxide FACILITATION OF 2-WAY AVOIDANCE

A Thesis Presented

by

Conchita Espino

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THE ROLE OF BRAIN AMINES IN THE CONDITIONED AVOIDANCE RESPONSE: CHLORDIAZEPoxide FACILITATION OF 2-WAY AVOIDANCE

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May, 1974
Three experiments are reported here which attempted to describe the mechanism of action of clorazepate (CDP) on the acquisition of conditioned avoidance response (CAR) in the shuttle box. It was hypothesized that the acquisition of two-way shuttle avoidance was facilitated by deactivation of the serotonergic system in the mesencephalon. The experiments demonstrated the following. 1. CDP facilitated the acquisition of CAR. 2. This facilitation by CDP was especially significant when stimulating grid shock levels were high. 3. Pre-treatment with CDP before shuttle-box tests did not weaken CDP-facilitation. 4. Alpha-methyl tryptamine, a serotonin agonist, did not attenuate CDP facilitation nor reverse CDP suppression of spontaneous activity. 5. Ritalin, an adrenergic agonist, also did not alter the CDP facilitation, but it did significantly increase inter-trial responding in combination with CDP as well as reverse CDP depression of spontaneous activity.

It is concluded that the facilitating effect of CDP on acquisition of the conditioned avoidance response is probably not due to any adrenergic mechanism in the mesencephalon. It is proposed that CDP's effect on turnover may not be due to interference with synaptic processes but rather with interference with axonal nerve impulse flow.
Acknowledgments

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Introduction

Benzodiazepines, such as chlordiazepoxide and oxazepam have a dual effect on behavior. The response decreasing (depressant action) and response increasing (disinhibitory action) properties of these drugs can be analyzed separately by the use of a procedure developed by Geller and Seifter (1960). In this procedure two alternating schedules of reinforcement are used. In the first a bar-press is reinforced by food on a VI-1 min. schedule. The second consists of a CRF schedule in which every bar-press is reinforced by both food and foot shock. Using this method, Margules and Stein (1968) demonstrated that the depressant action (decrease in rate of unpunished responses) undergoes tolerance after 3-4 doses, while the disinhibitory action (increase in the rate of punished responses) failed to show tolerance.

The mechanisms by which the Benzodiazepines exert their behavioral effect is not well understood. Pharmacological investigations indicate that these drugs affect monoamine turnover in the brain (Corrodi et al., 1971; Taylor and Laverty, 1969; Chase et al., 1970) and prevent the depletion of NE levels produced by electro-footshock (Taylor and Laverty, 1973).

Attempts have been made to relate the behavioral actions of the tranquilizers to their effects on monoamine
turnover. Stein et al. (1973) report evidence suggesting that the benzodiazepines exert their disinhibitory effects by reducing the activity of serotonin neurons in a behavioral suppressant "punishment system", and their depressant effects by reducing the activity of norepinephrine neurons in a behavioral facilitatory "reward system." Employing the Geller-Seifter "conflict test" they showed that the alpha-noradrenergic antagonist, phentolamine and the betanoradrenergic antagonist, propranolol, failed to release punished behavior, suggesting that norepinephrine is not essential in behavioral disinhibition. The role of serotonin (5-HT) in disinhibition of punished behavior is implicated by reports that p-chlorophenylalanine (PCPA), a serotonin synthesis inhibitor, releases punished behavior (Geller and Blum, 1970). Also, \( \alpha \)-methyltryptamine, a centrally active serotonin agonist (Vane et al., 1961), suppresses punished as well a non-punished behavior (Graeff and Schoenfeld, 1970). To further test the idea that serotonin is relatively more important in disinhibiting punished behavior than NE, Stein et al. (1973) administered via ventricular cannulas \( \beta \)-NE and 5-HT to two separate groups of rats. These rats had been pretreated with oxazepam and were compared to oxazepam-alone controls to examine which combination of substances would antagonize the oxazepam-induced release of punished behavior. These authors reported that 5-HT blocked
the oxazepam-induced release of punished behavior while l-NE potentiated it. In support of their hypothesis about the dual effects of benzodiazepines, Stein et al. (1973) presented evidence that the effect of the drug on NE turnover diminishes as the drug's behavioral depressant action undergoes tolerance, while the effect on serotonin turnover remained unchanged.

It is difficult to determine the role of NE in behavior maintained by negative reinforcement in studies employing α and β-blockers like phentolamine and propranolol since these are primarily peripheral blockers and their central effects are not well understood. Furthermore, propranolol, although by itself does not have disinhibitory effects in the conflict test, does potentiate the disinhibitory effects of CDP (Sepinwall, Grodsky, Sullivan and Cook, 1973). Moreover, the findings of Stein et al. (1973) that the benzodiazepines result in a decrease in NE turnover in the midbrain and that this effect diminishes with chronic doses must be interpreted in light of the technique used. These authors showed that in the midbrain-hindbrain region of the rat, the turnover of intraventricular administration of [³H]5-HT was reduced with administration of oxazepam. When diencephalon-forebrain sections were examined, turnover was not affected by oxazepam. It is possible that the failure of Stein et al. (1973) to find drug effects on turnover
in other areas besides the midbrain-hindbrain region can be interpreted by the results of Aghajanian and Bloom (1967) who reported that the greatest accumulation of intraventricular injected tritiated serotonin was found in the midbrain-hindbrain areas, and for the most part in nerve endings and axons. Therefore since tritiated serotonin is not taken up by diencephalon-forebrain structures as well as midbrain-hindbrain structures, it is possible that CDP effects on diencephalon-forebrain 5-HT turnover cannot be detected as well by this technique. Nevertheless, the benzodiazepines do lead to a decrease in norepinephrine turnover in the midbrain, and this decrease in turnover undergoes tolerance. This decrease in norepinephrine turnover can account for the behavioral depressant effect of the benzodiazepines which also undergoes tolerance.

However, the disinhibitory effects of the benzodiazepines, while distinct from the sedative effects and independent of NE turnover in the midbrain as Stein et al. (1973) have suggested are not necessarily independent of NE turnover changes in other brain areas. Other authors (Lidbrink et al., 1973), employing a different technique for measuring turnover from that of Stein et al. 1973) report reduction of NE and 5-HT turnover in cortical areas after i.p. administration of various benzodiazepines. In
addition, Taylor and Laverty (1973) showed that the greatest blockade of NE turnover by CDP was observed in the cerebral cortex.

It seems clear that although the sedative properties of Benzodiazepines and other minor tranquilizers are probably produced by their blockade of NE turnover in the midbrain, their effects on behavior under the control of aversive stimulation cannot as yet be solely attributed to the action on 5-HT in the midbrain; it may be due to NE turnover in the cortex.

In the present study we examined the effects of the Benzodiazepines on brain amines in the two-way avoidance paradigm in order to determine the importance of NE and 5-HT in disinhibition of behavior. Several authors (Sachs et al., 1966; Stiner et al., 1967; Taber et al., 1967) have reported that CDP facilitates two-way active avoidance. Pilot studies in our laboratory also suggested that at high shock levels the suppressant effects of punishment interfere with the acquisition of two-way avoidance and that CDP by reducing the suppressant effects of punishment aids in the acquisition of the avoidance response.

Two techniques were employed in order to assess the mechanism by which the drug affects the conditioned avoidance response (CAR). In the first experiment the effect of chronic dosages was compared to acute administration of
CDP in an effort to isolate depressant effects from the disinhibitory effects. Disinhibition was found to be independent of the depressant effect. According to Stein et al. (1973), 5-HT agonists should reverse the facilitation effects of CDP in the shuttle box, while a NE agonist should be without effect on the shuttle box. On the other hand, if the blockade of NE turnover produced by the benzodiazepines in cortical areas (Taylor and Laverty, 1973) is critical for this response disinhibition, then a drug reversing the pharmacological effects of CDP in an adrenergic system should reverse the CDP-induced facilitation. Methylphenidate (Ritalin), an adrenergic agonist, and 5-methyltryptamine (\(\text{5-MT}\)), a serotonergic agonist, were used to test the role of NE and serotonin respectively in disinhibition of shuttle avoidance.

Experiment 1

Method

Subjects

The subjects were forty-five male Sprague-Dawley rats, 120-150 days old with an initial average weight of 326 g. All animals were individually housed in wire mesh cages and given access to ad libitum food and water. The animals were assigned to groups so that weight was counterbalanced.

Apparatus

A two-way shuttle-box (Lehigh Valley Electronics
model #147-X39) was employed. The two compartments were separated by a sheet aluminum walk-through divider with an opening 6 x 6 cm. General illumination was provided by a dimmed room light. The CS was a tone emitted from a Sonalert on the center of the ceiling of the box. The CS-US interval was 10 seconds. Shock was provided by a Lehigh Valley solid state shoker/scrombler model #133-33. The shock intensity varied according to group (0.5, 1.0, and 2.0 ma). A trial began with the presentation of the shock during which the CS continued to be presented. A crossing during the 10 second CS-US interval constituted an avoidance and initiated the intertrial interval. A crossing after the onset of shock constituted an escape and also initiated the next intertrial interval. If no escape was made within 30 seconds the shock and the tone both terminated. Trials were presented on a variable schedule with a mean intertrial interval of 60 seconds. Any intertrial response (ITR) made during this time were recorded but did not initiate a new trial. Trials, number of shocks, ITR's and number of escapes were recorded for each 20 trial session.

Procedure

The subjects were divided into nine groups. Three control groups, three CDP-acute groups and three CDP-chronic groups. Each group was tested at one of the three
shock levels.

The chronic group were pretreated for ten consecutive days with 15 mg/kg of CDP, 15 mg/cc i.p., and then were given this same dose one half hour before testing for the next five days. The acute group were treated in the same way except that during the ten day pretreatment period they were given a similar volume of 0.9% saline. The control groups were pretreated with saline and given saline one half hour before the five testing sessions. On the initial test day all animals were given a 5 minute adaptation period in which activity was measured by the number of crossings made. Each animal was given a 20 trial session each day for five consecutive days.

Results

Acquisition of two-way shuttle box was significantly affected by shock level (Fig. 1). An analysis of variance showed a significant interaction for groups across shock levels (p<.05). It can be seen from Figure 1 that animals administered saline showed a decreasing number of avoidance responses as shock level increased. The differences between the saline group at .25 ma and at 1 ma were significantly different (p<.05). Animals receiving CDP both in the pretreated and non-pretreated group significantly increased the number of avoidance responses as shock level increased from .25 to .5 ma. Furthermore, Figure 1
Figure 1. Effects of pretreatment with CDP on avoidance responses at three shock levels.

(S-S) = pretreated with saline, tested with saline
(S-D) = pretreated with saline, tested with CDP
(D-D) = pretreated with CDP, tested with CDP.
shows that CDP administered before each session, at shock levels of 0.5 and 1.0 ma significantly increased the number of avoidance responses (p<.05). This effect of CDP remained even if Ss were pretreated for ten days before the five drug testing sessions. On the other hand, CDP did not facilitate shuttle avoidance at low shock levels nor did pretreatment with CDP facilitate avoidance at low shock levels. In fact, figure 1 shows that both pretreated and non-pretreated subjects avoided less than controls but this difference was not significant.

Figure 2 shows that intertrial responses decreased as shock level increased for all groups. An analysis of variance showed that the differences between shock levels and interaction effects were significant, but no significant group differences were found.

Experiment 2

Method

Subjects

The subjects were twenty-five male Sprague-Dawley, 120-150 days old with an initial average weight of 342 g. All rats were obtained and housed in the same way as in Experiment 1.

Apparatus

The two-way shuttle box was the same as in Experiment 1. An activity box consisting of a cylinder 62 cm. in diameter and 42 cm. deep with a mesh floor (Lehigh Valley activity
Figure 2. Effects of pretreatment with CDP on inter-trial responses during acquisition of avoidance responses.
MEAN INTER-TRIAL RESPONSES

SHOCK LEVEL (mA)

(D-D)

(S-D)

(S-S)
model #145-03) was used. Counters provided a record of the animal's activity as it interrupted light beams that are detected by photocells located along the perimeter of the cage. The box was kept in a quiet, darkened room away from the recording and programming apparatus.

**Procedure**

Five groups of 5 rats each were employed. The groups consisted of a saline and CDP alone controls which were compared to three groups receiving α-methyltryptamine in combination with CDP. All drugs were administered one half hour before each session. CDP was always administered at a dose of 15mg/kg while α-methyltryptamine was varied from 3 mg/kg to 12 mg/kg.

On the first day animals were placed in the activity box for 15 minutes. On the following five days all animals were tested as in Experiment 1 in the two-way shuttle box.

**Results**

Administration of increasing doses of α-methyltryptamine were unable to reverse the facilitatory effect of CDP on shuttle avoidance (Figure 3). An analysis of variance showed that there were no significant differences between any of the α-MT and CDP groups and the CDP-alone group, although all four groups differed significantly from saline controls (p<.05). Furthermore, no differences were found
Figure 3. Effects of $\alpha$-MT on CDP facilitated active avoidance.
% AVOIDANCE RESPONSES

- CDP
- CDP & α-MT (3 mg/kg)
- CDP & α-MT 6 mg/kg
- CDP & α-MT 12 mg/kg
- SALINE

DAY

1 2 3 4 5
between the various doses of α-MT.

Figure 4 shows that α-MT was also unable to reverse the depressant effects of CDP in the activity box. A Dunnett test showed that all CDP groups were significantly different from the saline groups (p < .05). In fact, a t-test showed that at the 12 mg dose of α-MT in combination with CDP a significant further depression of activity was found when this group was compared with the CDP-alone group (p < .05).

Experiment 3

Method

Subjects

The subjects were twenty male Sprague-Dawley rats, 120-150 days old with an initial average weight of 334g, obtained and housed in the same way as in Experiment 1.

Apparatus

The same equipment as in Experiment 2 was used.

Procedure

Four groups of 5 animals each receiving either, saline, CDP, Ritalin, and CDP+Ritalin were placed in the shuttle box for five sessions preceded by a 15 minute test in an activity box on the day before the first shuttle box session. All drugs were administered one half hour before each of the six testing periods. The injections were given i.p. at the following doses: CDP, 15mg/kg; Ritalin, 3mg/kg.
Figure 4. Effects of $\alpha$-MT on inhibition of spontaneous activity by CDP.
Results

Figure 5 shows the effect of Ritalin alone and in combination with CDP on avoidance behavior. It can be seen that Ritalin administered with CDP did not reverse the CDP facilitation of two-way shuttle avoidance nor did it have a significant effect by itself.

Figure 6 shows that the combination of CDP and Ritalin did have an effect on intertrial responses. An analysis of variance showed that the differences were significant (p<.05). However a t-test between the saline and ritalin alone groups showed that Ritalin by itself did not significantly increase intertrial responses. Figure 6 also shows that CDP alone increases intertrial responses and that CDP+Ritalin further increases intertrial responses. T-tests between saline and CDP-alone groups, and CDP-alone groups and CDP+Ritalin groups showed that these differences were significant (p<.05)

Spontaneous activity normally depressed by CDP was significantly reversed by administration of Ritalin to CDP treated rats (Figure 7). Comparisons of Figures 6 and 7 shows that the Ritalin-alone group, while unable to increase intertrial responses (Figure 6) was most effective in increasing spontaneous activity (Figure 7). Although this increase in spontaneous activity was significantly different from CDP alone a t-test showed it was not significantly different from controls.
Figure 5. Effects of Ritalin alone and in combination with CDP on active avoidance.
Figure 6. Effect of Ritalin alone and in combination with CDP on intertrial responses during acquisition of avoidance responses.
Figure 7. Effects of Ritalin alone and in combination with CDP on spontaneous activity.
Discussion

Chlordiazepoxide facilitated acquisition of two-way avoidance at high shock levels (0.5 and 1.0) (Figure 1). These findings may explain the previously discrepant reports of facilitation (Sachs et al., 1966; Henriksson and Jarbe, 1971); no effect (Kamano and Arp, 1967; Goldberg et al., 1974); and deficit (Chisholm and Moore, 1970). These findings are consistent with the results of Stein et al. (1973) and Cook and Davidson, (1973) who showed that the benzodiazepines are effective in releasing behavior under aversive control. We found that increasing the intensity of the aversive stimulus suppressed both the avoidance response and intertrial responses. (Figure 2). Experiment 1 showed that in the shuttle box the effect of high levels of aversive stimulation interferes with the acquisition of the adaptive response. Chlordiazepoxide, by lowering what are probably the emotional effects of the shock, facilitates the acquisition of the adaptive response. The results of Experiment 1 are also consistent with a set point of aversive stimulation hypothesis. According to this hypothesis, there is an optimum level at which aversive stimulation is effective in modifying behavior. As shown by the saline group, a further increase beyond this optimum level prevents the acquisition of an adaptive response. The effect of CDP seems to involve a change in this set
point so that the optimum level of aversive stimulation is shifted to higher shock levels. The decrease in intertrial responses with increasing shock levels indicates that the disruptive effect is accompanied by a decrease in activity.

Pretreatment with CDP although it attenuates the behavioral depressant effects, does not alter the facilitation of shuttle avoidance which occurs at higher levels of shock. This is consistent with the findings of Margules et al. (1968) that there is no decrease in the "anti-anxiety" activity of these drugs with chronic administration while there is recovery of behavioral depression. Pretreatment with CDP also did not have an effect at low shock levels suggesting that the lack of facilitation is not due to an overriding behavioral depression but to an independent effect of the drug which varies with levels of stimulation.

The greater number of intertrial responses for both CDP groups at the various levels of shock might suggest a general disinhibitory effect of CDP. Wuttke and Kelleher (1970) have proposed that the rate-enhancing effects of the Benzodiazepines in aversive situations are due to a general facilitation on low rates of responding rather than on a selective effect on behavior suppressed by punishment. Miczek (1973) has demonstrated in a conditioned suppression paradigm that Benzodiazepines do not in fact
enhance responding of low rates when the CS signaled response-independent reward, but does enhance responding when the CS signaled response-independent shock. In the present study we found that increases in intensity of the aversive stimulus resulted in decreased intertrial responses in control animals. This suggests that ITRs are also under the control of aversive stimulation and are not due to a general behavioral disinhibition.

In the first experiment we found that pretreatment with CDP, which according to Stein et al. (1973) normalizes the turnover rate of adrenergic neurons in the mesencephalon, does not prevent the facilitation of shuttle avoidance by CDP. This suggests that adrenergic neurons in the mesencephalon probably do not mediate the effect of the drug in shuttle box behavior. But, the role of NE in behavior under the control of aversive stimulation has been implicated by other investigators. Bliss et al. (1968) found that footshock increased the turnover of norepinephrine, while Taylor and Laverty (1973) found that the increase in turnover produced by footshock could be prevented by the benzodiazepines, especially in cortical areas. If tolerance to changes in NE turnover does not occur in the cortex in the same way as it does in the mesencephalon, then it would still be possible that CDP induced changes in cortical NE
would play a role in facilitating acquisition of avoidance behavior in the pretreated groups.

However our findings in Experiment 2 with Ritalin did not support this hypothesis. Ritalin, an adrenergic stimulant which is able to increase levels of adrenergic activity did not counteract the effect of CDP in the shuttle box. It is important to point out that Ritalin was effective in counteracting the effect of CDP in the activity measure. This implies that some pharmacological action of CDP is being reversed by Ritalin. It also should be pointed out that Ritalin, in spite of its effect on spontaneous activity, did not increase intertrial responses when administered alone. These results are consistent with those in Experiment 1 in which decreases in ITRs are found with increasing shock levels and support the hypothesis the ITRs are under the control of aversive stimulation. The greatest increases in intertrial responses were found with the group receiving both CDP and Ritalin. This finding suggests that some of the disinhibitory effects of CDP are being counteracted by the drug’s behavioral depressant effects which can be reversed by Ritalin without affecting the disinhibition effects.

Before discarding the hypothesis that NE plays a role in disinhibition of behavior and aids in the shuttle box at high shock levels, the possibility that CDP and Ritalin
are acting in two different areas should be tested. This can be done by measuring the turnover of NE after footshock with this drug combination in order to determine if Ritalin effectively reverses the effect of CDP on NE turnover in the cortex after footshock.

An effect of the benzodiazepines on serotonin turnover has also been suggested as the principal factor in release of punished behavior. Poschel and Nientman (1971) have suggested that the ascending 5-HT neurons could be considered as a non-reinforcement system selectively affected by the benzodiazepines. Stein et al. (1973) have also suggested a serotonergic punishment system deactivated by the benzodiazepines as the mechanism for the drugs disinhibition of behavior. Our findings, with -methyltryptamine, do not support this hypothesis. A second explanation for the findings would consider the hypothesis of Narahashi et al. (1971) that the benzodiazepines may be having their effect by causing a decrease in nerve impulse flow. Recently Lidbrink and Farnebo (1973) found that CDP does not mediate its effect on NE turnover in the cortex by affecting uptake or release at the adrenergic nerve terminal. These authors suggest that the drug may be having its effect on turnover by a decreased flow of neuronal impulses. It is possible that a similar mechanism
is instrumental for CDP's effect on mesencephalic 5-HT turnover. According to this formulation, the functional decrease in activity is controlled by axonal transmission, not by synaptic activity. This hypothesis would be consistent with our findings since neither an adrenergic nor a serotonergic receptor stimulant would be able to reverse the effect of the drug.
REFERENCES


