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Drug Alterations of Punished Responding After Chlordiazepoxide: Possible Screen for Agents Useful in Minimal Brain Dysfunction

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d-Amphetamine Methylphenidate Bupropion Punished responding Minimal brain dysfunction

THE attentional deficit disorder, hyperkinetic or Minimal Brain Dysfunction (MBD) syndrome is characterized by a number of behavioral difficulties including altered emotional and interpersonal processes, motor and perceptual-cognitive abnormalities and deficits in attention and impulse control [19]. While animal models for MBD have focused on reproducing the hyperkinetic characteristics of the syndrome [2, 7, 14], increased locomotor activity is, at best, an inconsistent symptom of MBD [19]. Other prominent symptoms, such as impulsivity, distractibility and short attention span [19] suggest that a deficit exists in impulse control. An appropriate model for MBD would not only provide a screening technique for drugs which could prove therapeutic, but could also be used to generate hypotheses concerning underlying brain mechanisms responsible for deficits in impulse control.

Responding in an operant task is reduced by punishment; further, chlordiazepoxide is known to antagonize the suppressed responding in this task [4,18]. In the present communication, a model has been developed to examine impulse control defined as the ability of rats to suppress responding that leads to mild punishment. Through its antipunishment action chlordiazepoxide results in a deficit in impulse control. While we do not suggest that this benzodiazepine treatment affects neurochemical systems identical to those responsible for MBD, the functional result in behavioral terms may be analogous. Indeed, benzodiazepines have been reported to have unfavorable effects on hyperkinetic children [20,22]. Central stimulants and certain antidepressant drugs can ameliorate symptoms of MBD while phenobarbital exacerbates the syndrome. Bupropion, which is being considered as a potential antidepressant [16,11], has a central stimulant effect in rats, but to our knowledge has not been tested for treatment of the hyperkinetic syndrome. Therefore, the present study examined the effectiveness of d-amphetamine, methylphenidate, imipramine and bupropion to antagonize the antipunishment actions of chlordiazepoxide and of phenobarbital to enhance these actions.

METHOD

Male Sprague-Dawley rats that were water deprived for 24 hours were trained to drink water in a paradigm similar to that described by Vogel *et al.* [18]. The rats were permitted 220 licks at the water spout before being removed from the test chamber. Most animals discovered the spout within two minutes. Eighteen hours later the rats were again placed in the apparatus. However, the twentieth lick of the spout now produced a 0.6 mA constant current electric shock between the water spout and the grid floor. Shock availability was maintained for two seconds and was then contingent upon every twentieth lick. The latency to complete the initial 20 licks was recorded as well as the total number of shocks received within three minutes after the first shock. This latter measurement reflects the capacity of the rats to control the impulse to drink. Latency between placement in the apparatus and the twentieth lick was also recorded so that it

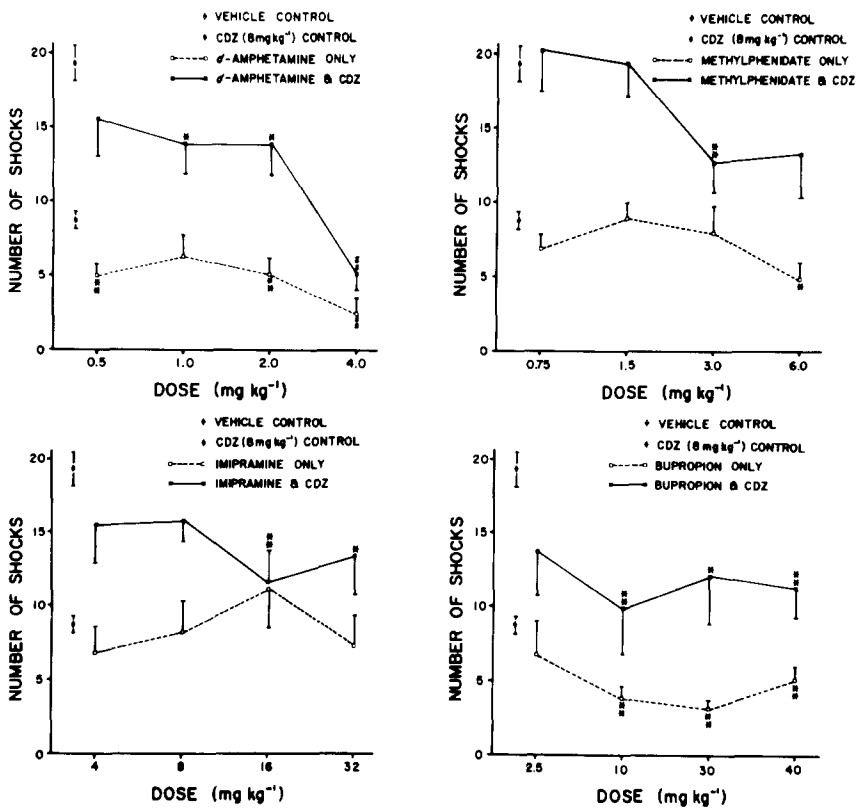

FIG. 1. Effects of varying doses of d-amphetamine, methylphenidate, imipramine, and bupropion on impulse control, measured as the number of shocks received in the conflict task. Curves are shown for either saline or CDZ pretreated rats. Isolated points represent scores for rats receiving saline only (open symbol) or CDZ (filled symbol). Significant differences from controls are indicated by filled-in circle ($^*p<0.05$; $^{**}p<0.01$).

could be determined whether a particular drug treatment caused general disorientation or motor impairment.

In the first experiment, the rats were injected IP 30 minutes prior to the punishment session with either saline (2 ml/kg) or chlordiazepoxide (CDZ, 8 mg/kg). d-Amphetamine, methylphenidate or bupropion were also administered 30 minutes prior to testing (CDZ first) while imipramine was administered 60 minutes beforehand.

Because of the possibility that drugs found effective in CDZ-treated rats might reduce licking by decreasing thirst [17], the experimental procedure was repeated with d-amphetamine (0.5 mg/kg) in rats given CDZ (8 mg/kg) but shock was eliminated, thus permitting the detection of reduced thirst due to the stimulant drug. In another experiment, it was determined whether d-amphetamine potentiated the depressant effects of CDZ, thereby weakening its disinhibitory action. Prior exposure to benzodiazepines has been shown to be an important factor in determining their effect in operant punishment procedures. It has been suggested that as tolerance develops to the depressant actions, maximal attenuation of the effect of punishment is observed [4]. Accordingly, 84 male Sprague-Dawley rats were tested in the lick suppression paradigm nine days after ten daily IP injections of CDZ (8 mg/kg) or saline. Acute drug treatments 30 minutes before testing consisted of saline plus saline, CDZ (8 mg/kg) plus saline, d-amphetamine (1 mg/kg) plus saline, or CDZ (8 mg/kg) plus d-amphetamine (1mg/kg).

Because phenobarbital is contraindicated for the hyperkinetic syndrome [3], the effects of this drug were also examined in this task. Rats received IP injections of either saline, CDZ (4 mg/kg), phenobarbital (5 mg/kg) or CDZ plus phenobarbital 30 minutes prior to the punishment session.

RESULTS

Figure 1 shows the effects of various doses of these four drugs on the number of shocks received by CDZ or saline-pretreated rats. Analyses of variance demonstrated that the number of shocks received by CDZ-pretreated rats was significantly reduced by the four drugs tested ($p<0.05$). d-Amphetamine and bupropion were the most potent.

In the test for hypodipsic effects of d-amphetamine, administration of this drug alone induced a small, but significant ($p<0.05$), decrease in licking. However, d-amphetamine given to rats pretreated with CDZ caused no change in the number of licks. Means and standard errors of the number of

TABLE 1

EFFECTS OF CHLORDIAZEPOXIDE (CDZ) AND PHENOBARBITAL ALONE AND IN COMBINATION ON SHOCKS RECEIVED

	Shocks Received (number/3 min)
Saline	9.6 ± 1.3
CDZ (4 mg/kg)	15.1 ± 3.1
Phenobarbital (5 mg/kg)	18.3 ± 2.8*
CDZ (4 mg/kg) + Phenobarbital (5 mg/kg)	26.0 ± 2.2*†

All groups contained eight rats except for the saline group which had five.

*Significantly different from saline, $p<0.01$, t-test.

†Significantly different from CDZ, $p<0.05$, t-test.

licks/three minutes for eight rats without shock delivery were as follows: saline/saline = 427±24; saline/d-amphetamine = 360±28; saline/CDZ = 510±36; CDZ/d-amphetamine = 530±20. Therefore, the finding that d-amphetamine antagonized the CDZ increase of punished responding cannot be explained by a simple loss of motivation to drink water. It is possible that the fewer shocks taken by animals that received only d-amphetamine could in part be related to a change in thirst.

In the test for tolerance to the disinhibitory effects of CDZ and the possibility for interaction of this tolerance with d-amphetamine, previous chronic treatment with CDZ had no influence. The mean (±s.e.) number of shocks taken was 4.5±0.7 for the saline/saline condition, 15.7±2.9 for the CDZ/saline, 2.4±0.3 for d-amphetamine/saline and 8.1±2.2 for CDZ/d-amphetamine. d-Amphetamine significantly attenuated CDZ increases of punished licking in the chronic CDZ-treated rats ($p<0.01$). This result suggests that the action of amphetamine was not due to an interaction with the acute depressant effects of CDZ.

As shown in Table 1, phenobarbital increased punished licking. Furthermore, the effects of phenobarbital were additive to those of CDZ.

DISCUSSION

The data show that the CDZ increase in responding during punishment is antagonized by those drugs that produce central stimulation (d-amphetamine, methylphenidate, and bupropion) and by imipramine. Such results are consistent with reports indicating that d-amphetamine can enhance the suppressive effects of punishment [8] and antagonize the punishment attentuating effect of CDZ [6]. Furthermore, phenobarbital enhanced the deficit in impulse control. These findings are consistent with the actions of these drugs in MBD and suggest that the CDZ change in punished responding serves as a pharmacological model of one symptom of MBD.

The data are consistent with reports showing improved cognitive performance in normal children who have been administered d-amphetamine [12]; saline-pretreated rats given d-amphetamine or methylphenidate showed "improved performance" in that the number of shocks taken was decreased by these drugs.

Shaywitz [13] has suggested that an animal model for MBD should satisfy the following four criteria: (1) the animal model must mimic the central features of MBD; (2) the human and animal syndromes must have similar pathogeneses; (3) the animal syndrome must be evident during early development; and (4) stimulants (and other drugs found effective in MBD) must ameliorate the animal syndrome. Insofar as persistence in punished responding may be analogous to lack of impulse control, the present model mimics one of the central features of MBD. Because of the heterogeneity of the clinical population, it may be impossible to develop one animal model that encompasses all of the features of MBD; therefore, criterion one, which may be too ambitious, is partially met by this animal model. Criterion two seems less of a criterion than an aim of the modelling process. Because adult animals were used, the present paradigm fails to satisfy criterion three. However, the procedure used assesses a behavioral capacity that is maturationally linked. Given the results in adult rats, it would be of interest to know whether central stimulants would reverse the deficiency of young rats to inhibit punished responses [1,15]. Nevertheless, criterion three might not be essential because there is evidence that some childhood symptoms of MBD, such as attentional deficits, persist into adulthood [9,21]. This model clearly satisfies criterion four. Therefore, we suggest that the paradigm described may provide a useful model for testing drugs potentially effective for the impulsivity seen in MBD. In this respect, the present data are consistent with reports of the potencies of d-amphetamine, methylphenidate, and imipramine in the treatment of MBD and also suggest the potential effectiveness of bupropion in this syndrome.

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