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Amphetamine Potentiation of Anti-Conflict Action of Chlordiazepoxide

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LERNER, T , J FELDON AND M S MYSLOBODSKY *Amphetamine potentiation of anti-conflict action of chlordiazepoxide* PHARMACOL BIOCHEM BEHAV **24**(2) 241–246, 1986 —A modified Geller-Seifter paradigm was employed to test in male albino rats the effects of subthreshold doses of amphetamine and chlordiazepoxide (CDP), administered separately or in combination, on shock induced suppression of food-reinforced lever-pressing MK-801, a newly synthesized sympathomimetic with anxiolytic and anticonvulsant properties, was also tested dl-Amphetamine in doses of 0 1, 0 2, 0 3, and 1 0 mg/kg had no anxiolytic nor anxiogenic effects, but at 1 0 mg/kg it increased non-conflict responding CDP in doses of 0 1, 0 2, and 0 4 mg/kg had no significant effect on conflict and non-conflict responding CDP in the dose of 0 8 mg/kg tended to increase conflict responding Coadministration of amphetamine (0 2 mg/kg) and CDP (0 4 mg/kg) had a significant anti-conflict effect MK-801 at 50 μg/kg and 100 μg/kg caused a significant increase in non-conflict responding MK-801 at 50 μg/kg exerted also a significant anti-conflict effect The disinhibitory effects of amphetamine coadministered with CDP were discussed in terms of a possible enhanced noradrenergic or dopaminergic activity and their interaction with GABA neurotransmission at GABA-benzodiazepine coupled sites

dl-Amphetamine Catecholamines Chlordiazepoxide HCl Benzodiazepines MK-801
Locus-coeruleus Anxiety Punishment Anti-conflict test Rats

REDMOND *et al* [37] hypothesized that anxiety results from chronic overactivity of the noradrenergic neurons originating from the locus coeruleus (LC)-norepinephrine (NE) system, or hyperactivity of target neurons, and that the benzodiazepines-produced augmentation of punished responding is mediated by the antagonism of LC activity [38] A similar suggestion has been forwarded by Gray [19] on the basis of findings that dorsal noradrenergic bundle lesions exert anti-anxiety effects as indicated by the alleviation of punishment induced suppression of responding in rats This reasoning implies that the psychomotor stimulant, amphetamine, which is known to enhance the release and to reduce the reuptake of NE [20] should cause or potentiate anxiety related behavioral responses Furthermore, benzodiazepines would be expected to counteract these effects of amphetamine While numerous findings are consistent with these predictions [11, 15, 22], there are puzzling observations of amphetamine induced potentiation of benzodiazepine action in various behavioral paradigms [3, 14, 41, 43, 44]

A related paradoxical effect of amphetamine is its anticonvulsant action The correlation between the anxiolytic potency of benzodiazepines and their ability to suppress metrazol-induced seizures is well documented [46] In contrast, anxiety inducing substances are broadly classified as proconvulsants or genuine convulsants Therefore, amphetamine would be expected to augment the susceptibility to metrazol-induced convulsions However, this is found only with high doses of the drug (10–15 mg/kg), whereas low doses (1–4 mg/kg) typically enhance resistance to seizures [25] and potently suppress photoconvulsive discharges [5, 26, 34] This effect can not be attributed to enhanced LC activity Rather, based on studies of Graham and Aghajanian [18], the anticonvulsant properties of amphetamine may be related to reduced "LC tone " Low doses of systemically administered amphetamine have been found to augment the rate of spontaneous firing of hippocampal neurons [24] Since hippocampal cells receive inhibitory influences from the LC, this result was interpreted as suggesting that amphetamine reduced LC-mediated control over some target neurons Assuming that receptors mediating this effect are identical or similar to the noradrenergic alpha-2 receptor, one could argue that amphetamine, like clonidine [6, 38, 39], might have anxiolytic rather than anxiogenic properties

The present study examined this possibility by testing the effects of subthreshold doses of amphetamine and chlordiazepoxide, administered separately or in combination, on punished responding in a Geller-Seifter [17] conflict paradigm In addition, dose-response effects of amphetamine were compared with those of a newly synthesized sympathomimetic, MK-801 which has been reported to have pronounced anticonvulsant and anxiolytic properties [8,9]

The Geller-Seifter procedure is a multiple operant schedule consisting of periods of variable interval reinforcement alternating with periods of continuous reinforcement in which each response produces both reinforcement and footshock (the conflict period) Response rate in the conflict period which is normally suppressed by footshock is further suppressed by amphetamine and is increased by anxiolytics (e g , [11]) In the present modified procedure the reinforce-

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TABLE 1

THE EFFECT OF MK-801 ON CONFLICT AND NON-CONFLICT RESPONSES IN THE GELLER-SEIFTER PARADIGM

Test Drug	Dose (IP)	# of Replic	Non-Conflict Responses (mean ± SEM) Saline	Drug	F Values	Conflict Responses (mean ± SEM) Saline	Drug	F Values
MK-801	50 μg/kg	4	54 ± 4 1	113 ± 8 7	16 91*	10 57 ± 1 6	36 ± 5 3	12 6*
MK-801	100 μg/kg	3	60 ± 4 2	114 ± 8 3	49 11‡	9 05 ± 1 9	31 8 ± 3 7	4 56
MK-801	250 μg/kg	2	67 ± 9 6	20 ± 8 6	6 03†	5 06 ± 1 2	2 08 ± 0 2	17 89†

MK-801 was tested against its saline vehicle After injection rats were placed in the Skinner-box and allowed to bar-press for food in the CONF and NCONF segments of the session Values represent the mean (±SEM) of the total number of responses during the CONF and NCONF segments of the paradigm with number of replications specified A two-way ANOVA for repeated measurements composed of drug effects (drug vs saline) and replications was carried out

0 05<†p<0 10, *p<0 05, ‡p<0 01

ment schedule remained constant throughout the pre-conflict and conflict periods (see Procedure)

METHOD

Subjects

The subjects were 16 male Wistar rats (Tel-Aviv University Medical School, Israel) of about 90 days of age, weighing approximately 300 g at the start of the experiment They were housed individually under reversed cycle lighting for the duration of the experiment

Apparatus

Four standard two-lever Campden Instruments (CI 460) Skinner boxes, each enclosed in a sound-attenuating box The right-hand lever was absent Reinforcement for responding on the left-hand lever was one 45 mg precision food pellet (Campden Instruments Ltd) The grid floors were connected to Campden Instruments constant current shock generator (521/C) and scrambler (521/S) A Rockwell-AIM 65 microprocessor was used for equipment programming and data recording

Drugs

The following compounds were used dl-Amphetamine (Amph) (Smith, Kline and French), Chlordiazepoxide HCl (CDP) (Hoffman-La Roche, Inc), and MK-801 (courtesy of Dr Clineschmidt, Merck Institutes), all dissolved in saline dl-Amph, CDP, and MK-801 were each administered IP 10, 20, and 30 min, respectively, prior to the drug sessions

Training

Two weeks prior to the start of the behavioral training rats were food deprived, and received approximately 8 g of rat chow per day Animals were maintained at approximately 80% of their free body weight throughout the experiment Water was freely available in the home cages All rats were handled for approximately 30 sec a day for one week before the beginning of the experiment Following this period rats received magazine training and shaping until their responding stabilized on a variable interval (VI) 60 sec schedule Each VI session length was 57 min The two lights above the two levers (the right lever was retracted) were on throughout, and the animals were run for 7 days a week After 3 months of such VI sessions, animals were run under a modified Geller-Seifter procedure [36] The traditional Geller-Seifter conflict test is a mixed schedule consisting of baseline (non-conflict) components of VI reinforcement alternating with conflict components in which both food and shock are delivered on an FR schedule Thus, the transfer from the baseline responding component to the signalled conflict component coincides with a change in the schedule on which the animals are reinforced Consequently, an increase in responding during both the baseline and the signalled conflict segment is difficult to interpret Also, drugs which might affect incentive motivation may influence the two components differently, since they differ in reinforcement density Therefore, in our modified procedure the reinforcement schedule (VI-60 sec) remained constant throughout the baseline and the signalled conflict components This allowed a direct comparison between the baseline response rate and the conflict component response rate

Each test session lasted 57 min and consisted of 5 segments of 9 min food VI-60 sec (non-conflict—NCONF) and 4 intrusion periods of 3 min food and shock VI-60 sec (conflict—CONF) The intrusion period was signalled by the house light flashing at 2 Hz during the entire period The first shock was delivered upon the first response emitted during this period and for the rest of the 3 min period it was delivered on a VI-60 sec schedule, independently of the VI-60 sec food schedule, which continued throughout the non-conflict and conflict periods

Shock duration was 0 5 sec, shock level was initially 0 1 mA, and was increased individually for each subject so that the degree of suppression during the CONF period, measured in terms of suppression ratio, ranged between 0 10 and 0 25 The suppression ratio was obtained by dividing the number of lever presses during the 3 min CONF period by the sum of responses in the CONF period added to the number of responses during the 3 min of the NCONF period preceding the CONF period Since the experiment lasted for several months, it was necessary, as in previous similar experiments [36], to adjust occasionally the shock level in order to maintain the suppression ratios in the 0 10–0 25 range This resulted in some fluctuations in animals' responding, particularly during CONF segments The actual shock levels ranged between 0 1 mA and 0 25 mA

TABLE 2

THE EFFECTS OF CDP, Amph AND THEIR COMBINATIONS ON CONFLICT AND NONCONFLICT BEHAVIOR IN THE GELLER-SEIFTER PARADIGM

Test Drugs	Dose (IP)	# of Replic	Non-Conflict Responses (mean ± SEM) Saline	Drug	F Values	Conflict Responses (mean ± SEM) Saline	Drug	F Values
CDP	0 1 mg/kg	2	42 ± 4 1	44 ± 4 3	0 45	5 18 ± 2 3	6 28 ± 0 9	1 09
CDP	0 2 mg/kg	2	31 ± 3 9	41 ± 3 6	1 91	5 03 ± 0 8	4 15 ± 1 02	0 29
CDP	0 4 mg/kg	2	46 ± 7 9	37 ± 7 0	0 24	8 46 ± 2 2	5 82 ± 1 3	2 02
CDP	0 8 mg/kg	3	40 ± 4 7	44 ± 7 5	0 18	5 70 ± 1 9	11 14 ± 3 6	6 54†
Amph	0 1 mg/kg	2	48 ± 5 7	47 ± 6 8	0 04	8 59 ± 1 3	8 68 ± 1 8	0 01
Amph	0 2 mg/kg	3	30 ± 2 7	43 ± 6 5	3 16	4 64 ± 1 0	5 2 ± 1 09	1 87
Amph	0 3 mg/kg	3	33 ± 3 1	48 ± 4 9	4 16	9 08 ± 2 3	14 66 ± 3 1	1 31
Amph	1 0 mg/kg	1	34 ± 7 8	69 ± 8 6	14 2*	1 06 ± 0 9	4 75 ± 2 5	4 69
Amph + CDP	0 1 mg/kg + 0 1 mg/kg	2	37 ± 4 9	42 ± 3 5	3 53	7 0 ± 1 9	14 56 ± 2 9	1 23
Amph + CDP	0 2 mg/kg + 0 2 mg/kg	1	33 ± 7 2	40 ± 7 2	3 12	2 31 ± 0 8	9 31 ± 4 0	3 47
Amph + CDP	0 2 mg/kg + 0 4 mg/kg	1	34 ± 1 5	46 ± 10 2	5 43	9 12 ± 3 0	29 12 ± 11 9	232 7§
Amph + CDP	0 3 mg/kg + 0 8 mg/kg	1	30 ± 12 8	48 ± 6 0	48 7‡	9 18 ± 3 1	29 36 ± 6 1	13 7*

Amph , CDP and their combinations were tested against their saline vehicle After injection rats were placed in the Skinner-box and allowed to bar-press for food in the NCONF and CONF segments of the session

Values represent the means (±SEM) of the total number of responses during the NCONF and the CONF segments of the paradigm, with the number of replications specified A two-way ANOVA for repeated measurements composed of drug effects (drug vs saline) and replications was carried out

$0\ 05<\dagger p<0\ 10$, $*p<0\ 05$, $\ddagger p<0\ 01$, $\S p<0\ 001$

Subjects were run for 50 stabilization test sessions and were then divided into 4 drug treatment groups matched for response rates and suppression ratios Amphetamine (2 replications of 0 1 mg/kg, 3 replications of 0 2 mg/kg, 3 replications of 0 3 mg/kg, and 1 replication of 1 0 mg/kg), CDP (2 replications of 0 1 mg/kg, 2 replications of 0 2 mg/kg, 2 replications of 0 4 mg/kg, and 3 replications of 0 8 mg/kg), Amphetamine + CDP (two replications of 0 1 mg/kg Amph + 0 1 mg/kg CDP, one replication of 0 2 mg/kg Amph + 0 2 mg/kg CDP, one replication of 0 2 mg/kg Amph + 0 4 mg/kg CDP, and one replication of 0 3 mg/kg Amph + 0 8 mg/kg CDP), MK-801 (4 replications of 50 μg/kg, 3 replications of 100 μg/kg, and 2 replications of 250 μg/kg)

All animals were tested once daily and each animal was used as its own control Drugs were administered on every third day On the non-drug days rats were injected with 1 ml/kg of saline

For each subject, in each session, the number of responses were recorded in the four intrusion (conflict) periods, and in the 3 min period preceding each intrusion period Thus, the data submitted to analysis included eight scores per rat per session, consisting of the number of responses in the four 3-min conflict periods and in the four 3-min pre-conflict periods The data were analysed using a two-way analysis of variance (ANOVA) composed of two repeated measurement factors of drug (drug vs saline) and replications

RESULTS

MK-801 Effects

Table 1 presents the analysis of the effect of MK-801 compared with the saline controls MK-801 at the dose of 50 μg/kg had a significant effect on both the NCONF rate of responding, $F(1,3)=16\ 91$, $p<0\ 03$, and on the CONF response rate, $F(1,3)=12\ 6$, $p<0\ 04$ The injection of 100 μg/kg of the drug enhanced response rate during the NCONF period, $F(1,3)=49\ 11$, $p<0\ 007$, but failed to induce a significant increase in the CONF response rate, $F(1,3)=4\ 56$, $p>0\ 10$ At the dose of 250 μg/kg a suppressive effect on both the NCONF and the CONF response rates was observed These effects approached significance, $F(1,3)=6\ 03$, $p<0\ 10$, and $F(1,3)=17\ 89$, $p<0\ 07$, respectively

Amphetamine Effects

Table 2 presents the analysis of the effects of amphetamine Doses of 0 1, 0 2 and 0 3 mg/kg were subthreshold for affecting either the CONF or the NCONF responding At the dose of 1 0 mg/kg the drug exerted a significant facilitatory effect on the non-conflict performance, $F(1,3)=14\ 2$, $p<0\ 04$, while failing to disinhibit CONF responding, $F(1,3)=4\ 69$, $p>0\ 10$ On the whole, there was a dose-response trend in the effect of amphetamine on NCONF behavior, i e , the higher the drug dose, the higher

the relative increase in bar-pressing compared to the saline baseline during the NCONF periods

CDP Effects

CPD in doses of 0 1, 0 2, 0 4, and 0 8 mg/kg was subthreshold for eliciting any reliable change in either the NCONF or the CONF responding The effect of 0 8 mg/kg of CDP on CONF response rate approached significance, $F(1,3)=6\ 54$, $p<0\ 09$ (Table 2)

Amphetamine-CDP Coadministration

The Amph + CDP combination failed to affect significantly the response rate in either the NCONF or the CONF segments at the lower doses (0 1 mg/kg Amph + 0 1 mg/kg CDP, and 0 2 mg/kg Amph + 0 2 mg/kg CDP) At the higher dose (0 2 mg/kg Amph + 0 4 mg/kg CDP) the combination significantly increased the CONF response rate, while failing to change significantly the NCONF performance, $F(1,3)=232\ 7$, $p<0\ 001$, and $F(1,3)=5\ 43$, $p=0\ 10$, respectively At the highest combined doses (0 3 mg/kg Amph + 0 8 mg/kg CDP) there was a significant increase in bar-pressing in both the NCONF, $F(1,3)=48\ 7$, $p<0\ 007$, and CONF, $F(1,3)=13\ 7$, $p<0\ 04$, periods

DISCUSSION

The present results confirm those of Clenschmidt *et al* [9] demonstrating an anti-conflict action of MK-801, although the disinhibitory effect of the drug on punished responding was obtained along with an enhancement of non-conflict responding This difference may be due to differences in the mode of drug administration and the time of testing Clenschmidt *et al* [9] administered the drug orally and tested their animals 1 hr later

The anti-conflict effect of the widely used benzodiazepine, CDP, was marginal at the highest dose employed in the present study, which is consistent with findings of Rawlins *et al* [36] Amphetamine exerted neither anxiogenic nor anxiolytic action when administered alone These results appear inconsistent with findings showing that amphetamine depresses conflict responding [17, 21, 28, 31] and are most probably due to the procedural differences such as the use in our study of a different schedule of food and shock administration compared with the traditional Geller-Seifter procedure [17] or the intensity of the shock used [16,32] Under conditions of mild stress amphetamine is known to enhance responding such as bar-pressing for a dim light [4], or even a bright stroboscopic flash [34]

The central result of the present study is that amphetamine coadministered with CDP (both in subthreshold doses), potently disinhibited punished responding These findings are in line with several studies indicating agonistic interaction of amphetamine and the benzodiazepines Sansone [43] reported that several benzodiazepines (bromazepam exempted) caused a significant enhancement of stimulant-induced locomotion Amphetamine coadministered with pentobarbital or CDP to pigeons enhanced punished responding that exceeded the levels obtained with either of the drugs given alone [3]

The neuronal substrate of this interaction is obscure and cannot be determined from the existing data Amphetamine facilitates both noradrenergic and dopaminergic neurotransmission [2,20] Since dopamine has been implicated in anxiety [47], it is conceivable that this effect is mediated by enhanced DAergic activity The agonistic interaction between DAergic and GABAergic compounds is not uncommon It has been demonstrated [1,7] that the ability of some neuroleptics (which selectively inhibit D-2 receptors) to inhibit methylphenidate-induced gnawing in mice is reduced after pretreatment with diazepam and THIP Likewise, several studies revealed a considerable degree of anatomical coupling between benzodiazepine receptors and NE carrying terminals [13] even though the functional significance of this linkage remains poorly understood Sabato *et al* [42] found that along with a decrease by about 70% of the NE content of the cerebral cortex in the 6-OHDA treated rats there was a 20% decrease in benzodiazepine receptors labelling and a five-fold increase in their affinity Doble *et al* [13] found a 20% decrease in Bmax in the cerebellum of rats with no change in affinity after intracerebroventricular 6-OHDA These findings suggest that benzodiazepine sites are located on the NE input to the cortex or the cerebellum

In view of the contribution of GABA to the anti-conflict activity of various drugs, the disinhibitory effects of amphetamine coadministered with CDP could be attributed to enhanced NE modulatory action on neurotransmission at GABA coupled benzodiazepine sites This influence may be similar to that of stress which causes an increase in benzodiazepine receptors paralleled by reduced susceptibility to seizures [45] Indeed, NE released synaptically (by stimulation of the LC) or iontophoretically potentiated the responses of Purkinje cells to the inhibitory action of GABA The same doses of NE, by themselves, elicited little or no change in spontaneous firing of these cells [23,48] Similar changes were observed in the cerebral cortex [48]

Additional evidence favouring NE modulation of the GABA/benzodiazepine receptor complex comes from the recent findings demonstrating that the nM concentration of the beta-blocker, propranolol, inhibited GABA stimulated benzodiazepine binding [33], whereas GABA or benzodiazepine antagonists opposed the anti-petit mal action of amphetamine [35] Further circumstantial support for the possible anti-punishment effect of amphetamine comes from the demonstration that this drug augments beta-endorphin release [10], thereby producing an analgesic effect [27] The above biochemical and electrophysiological evidence may explain the behavioral pattern caused by the amphetamine-CDP combination However, much work is still needed to further clarify the significance and nature of this finding

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