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TRICYCLIC ANTIDEPRESSANT DRUGS: ATTENUATION OF EXCITATORY EFFECTS OF d-LYSERGIC ACID DIETHYLAMIDE (LSD) ON ACOUSTIC STARTLE RESPONSE

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Summary

At a dose of 5 mg/kg, the tricyclic antidepressant drugs chlorimipramine (CIMI), desipramine (DMI), imipramine (IMI), and chlordesipramine (C-DMI) all blocked the excitatory effects of a low dose (30 μg/kg) of LSD on the acoustic startle response in the rat. Over a dose range from 1-5 mg/kg, CIMI and DMI were about equally potent in blocking the LSD effect, despite the fact that both drugs actually increased brain levels of LSD. In contrast, α-methyl-p-tyrosine did not block the effect of LSD on startle. By themselves, DMI, IMI and C-DMI increased startle amplitude 20-30% whereas CIMI alone had no effect on startle. The ability of CIMI and IMI to block the excitatory effect of LSD on startle is consistent with the hypothesis that prior cessation of raphe cell firing caused indirectly by these drugs with no resultant change in 5-HT availability should pre-empt the ability of LSD to increase startle by directly inhibiting raphe cell firing and decreasing 5-HT availability. The finding that the other tricyclics also block the effect of LSD is not explained by that hypothesis. Results are discussed in terms of the serotonin hypothesis of the action of hallucinogenic drugs on behavior.

There is increasing evidence that the startle response in the rat is modulated by the central raphe-serotonin system. Electrolytic lesions of the midbrain raphe nuclei increase startle amplitude (10,18). Depletion of brain serotonin (5-HT) by the drugs parachlorophenylalanine (8) or parachloroamphetamine (13) also increase startle amplitude. Conversely, increased release of 5-HT shortly after administration of parachloroamphetamine (13) or direct infusion of 5-HT intraventricularly (19), or into specific areas of the brain (17) depress startle. Taken together, these results suggest that 5-HT inhibits startle or inhibits systems which are excitatory to startle.

Drugs which are hallucinogens in man alter startle amplitude in the rat, since low doses of LSD, DMT, psilocybin, mescaline and STP all have excitatory effects on startle (11,12,15,17). The finding that low doses of hallucinogens increase startle amplitude may be related to the fact that low doses of these drugs directly inhibit the spontaneous firing rate of midbrain raphe neurons

(3,4,7,21) and thereby decrease the release of 5-HT (16). Therefore, low doses of hallucinogens by reducing 5-HT availability may have the same functional effect as acute lesions of the raphe nuclei. In fact, LSD no longer has its excitatory effect on startle in animals with lesions of the midbrain raphe nuclei (10).

Unlike LSD, tertiary amine tricyclic antidepressants such as chlorimipramine (CIMI), which retard the synaptic inactivation of 5-HT by blocking 5-HT reuptake, tend to increase the availability of 5-HT (16). Paradoxically the tricyclic compounds are similar to LSD in that they also depress raphe cell firing rates (6,28). However, the depression of raphe cell firing by LSD and CIMI are mediated through different mechanisms. The depressant effect of CIMI appears indirect since it is dependent on 5-HT availability and can be prevented by prior 5-HT depletion via parachlorophenylalanine - PCPA (28). In contrast, the depressant effect of LSD on raphe cell firing is not altered by PCPA indicating that its action is independent of 5-HT (2). Moreover, the physiological consequences of LSD and CIMI on 5-HT transmission are also quite different. Thus LSD decreases the availability of 5-HT whereas CIMI at moderate doses (5 mg/kg) causes no net change in 5-HT availability. At high doses (20 mg/kg) CIMI actually increases 5-HT availability, even though raphe cell firing rates are depressed in all of these conditions.

These considerations suggest that the effects of LSD and CIMI on the startle response would be different or even antagonistic, despite the fact that both drugs depress raphe cell firing rates. If LSD increases startle by decreasing 5-HT availability through direct inhibition of raphe cell firing, then pretreatment with CIMI, which depresses raphe cell firing indirectly, should prevent LSD from increasing startle. That is, if LSD is given to an animal whose raphe cells have already been depressed by a mechanism which does not reduce 5-HT availability, LSD should have nowhere to act and hence be ineffective. By itself, moreover, CIMI should have no effect on startle, since at this dose (5 mg/kg) it causes no net change in 5-HT availability.

The purpose of the present study, therefore, was to test the effect on startle of CIMI alone and in combination with LSD. As comparison drugs, other tricyclic antidepressants (desipramine, chlordesipramine, imipramine) and the catecholamine synthesis inhibitor, α-methyl-p-tyrosine (AMPT) were tested also.

Methods

Animals. A total of 244 male albino Sprague Dawley rats (250-300 g) were used.

Apparatus. The apparatus has been described in detail elsewhere (33). Briefly, 5 stabilimeters were used to record the amplitude of the startle response. Each stabilimeter consisted of an 8 x 15 x 15 cm Plexiglas and wire mesh cage suspended between compression springs within a steel frame. Cage movement resulted in displacement of an accelerometer and startle amplitude was defined as the maximum accelerometer voltage that occurred during the first 200 msec after the startle stimulus was delivered. The stabilimeters were housed in a dark, ventilated, sound attenuated chamber, 1.1 m from a loud speaker. The startle stimulus was a 4000 Hz 90 msec 120 db tone having a rise-decay time of 5 msec. Background white noise was 46 db (General Radio Model 1551-C sound level meter - A Scale).

Startle Procedures. Table 1 shows the basic design used in all experiments. In each experiment 4 groups of 5 rats each were used. Each group was tested in 4 sessions using the various drug combinations listed in Table 1. Sessions were 2 days apart and involved the presentation of 90 tones at a 20

sec intertone interval immediately after the second drug injection. The first drug injection (pretreatment drug) was given 15 min earlier in the colony room. For the AMPT experiment the injection was given 1 hr earlier instead of 15 min.

The pretreatment drugs were chlorimipramine (CIMI) at doses of 1, 2, 4 or 5 mg/kg; desipramine (DMI) at doses of 1, 2, 4 or 5 mg/kg; imipramine (IMI) at 5 mg/kg; chlordesipramine (C-DMI) at 5 mg/kg; and α-methyl-p-tyrosine (AMPT) at 100 mg/kg. The dose of LSD was always 30 µg/kg (free base) diluted with distilled water. Thus the basic design was tested in 11 groups of 20 rats each. Each rat served as its own control with respect to each drug-combination whereas pretreatment drugs and the different orders across sessions varied between subjects.

TABLE 1

Basic Design of the Orders of Drug Injections Used in All Experiments

Group	Session 1	2	3	4
I	Saline-Water	Saline-LSD	Drug-Water	Drug-LSD
Ii	Drug-LSD	Drug-Water	Saline-LSD	Saline-Water
III	Drug-Water	Drug-LSD	Saline-Water	Saline-LSD
IV	Saline-LSD	Saline-Water	Drug-LSD	Drug-Water

LSD Assay Procedure. To determine whether CIMI or DMI altered the levels of LSD in the brain, rats were injected intraperitoneally with either saline (n = 8), CIMI (n = 8) or DMI (n = 8). Fifteen min later, all the rats received an i.p. injection of lysergic acid diethylamide [2-^{3}H(N)]-(30 µg/kg, 1.1 Ci/mM, New England Nuclear). Rats were killed by decapitation 15 min after the ^{3}H-LSD injection. Their brains were rapidly removed, weighed and frozen on dry ice. ^{3}H-LSD was extracted from brain homogenates according to the method of Axelrod et al. (5) as modified by Aghajanian and Bing (1); aliquots of final aqueous extracts were assayed for radioactivity using a Packard liquid scintillation counter.

Results

Effect of CIMI (5 mg/kg). Figure 1 shows the effects of LSD on startle in animals pretreated with CIMI (5 mg/kg) or saline. Following saline pretreatment, LSD had a marked excitatory effect on startle. In contrast, following CIMI pretreatment, LSD was much less effective in augmenting startle. CIMI by itself had no significant effect on startle. These conclusions were supported by an overall analysis of variance which revealed a significant effect of LSD ($F = 33.8$, $df = 1/16$, $p < .001$); no significant effect of CIMI ($F = 1.42$, $df = 1/16$, $p > .25$) but, most importantly, a significant Pretreatment Drug by Test Drug interaction ($F = 13.06$, $df = 1/16$, $p < .002$).

Effects of DMI (5 mg/kg). Figure 2 shows data comparable to those in Figure 1 in animals pretreated with DMI rather than CIMI. DMI also markedly reduced the usual excitatory effect of LSD which was highly significant (F interaction = 15.77, $df = 1/16$, $p < .001$). DMI by itself increased startle by about 30% ($t = 2.68$, $df = 19$, $p < .02$).

Effects of various doses of CIMI and DMI. Figure 3 shows a summary of the results from experiments in which the ability of various doses of CIMI or DMI to block the effects of LSD on startle were tested. In each experiment the percent blocking shown on the ordinate was calculated in the following way [(% LSD effect after saline) minus (% LSD effect after CIMI or DMI at the

particular dose)]/(% LSD effect after saline) X 100.

For both CIMI and DMI, higher doses produced a greater attenuation of the excitatory effect of LSD. This was supported by a significant Dose by Pretreatment Drug by Test Drug interaction ($F = 11.56$, $df = 3/32$, $p < .001$ for CIMI and $F = 7.62$, $df = 3/32$, $p < .01$ for DMI). However the dose-response blocking curves of DMI and CIMI did not differ statistically from each other.

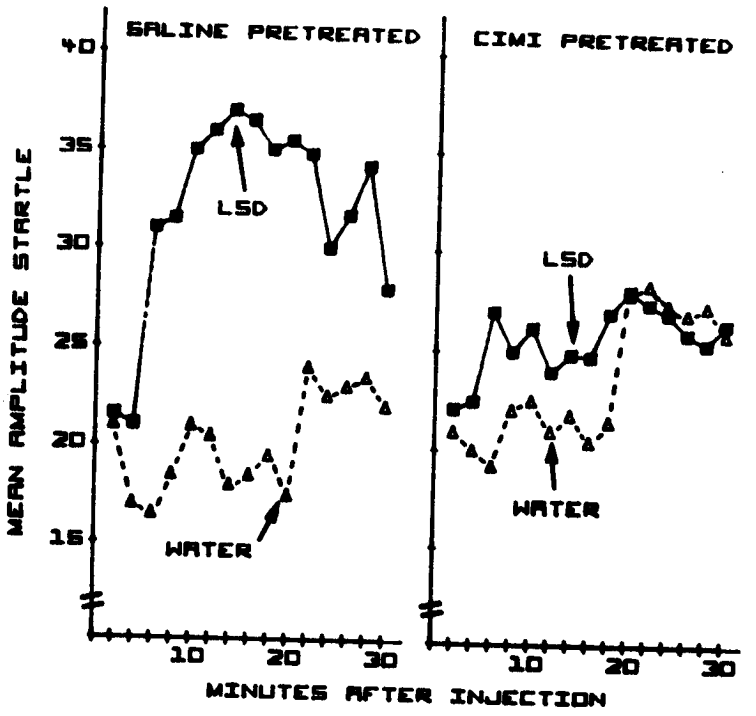

FIG. 1

Mean amplitude startle response over successive 2-min periods after injection with either LSD or water in rats pretreated 15 min earlier with either saline (left panel) or 5 mg/kg chlorimipramine (right panel).

Effects of IMI and C-DMI. Both IMI and C-DMI at doses of 5 mg/kg attenuated the excitatory effect of LSD on startle. For IMI the % block was 87% ($p < .001$) and for C-DMI the block was 91% ($p < .05$). By themselves IMI increased startle by about 21% ($p < .05$) and C-DMI by about 29% ($p < .05$). Thus all four tricyclic antidepressant drugs at the 5 mg/kg dose were about equally effective in blocking the excitatory effect of LSD on startle.

Effect of AMPT. Figure 4 shows the effects of LSD on startle after pretreatment with either saline or AMPT. In contrast to the antidepressant drugs, AMPT did not block the excitatory effect of LSD on startle at a dose and pretreatment interval that essentially eliminates the excitatory effects of high doses of d- or l-amphetamine on startle (14).

Effects of CIMI and DMI on Brain Levels of Radioactive LSD. Table 2 shows that both DMI and CIMI significantly increased the amount of ^{3}H-LSD re-

covered from brain at the time in which both drugs blocked the excitatory effect of LSD on startle in the previous experiments.

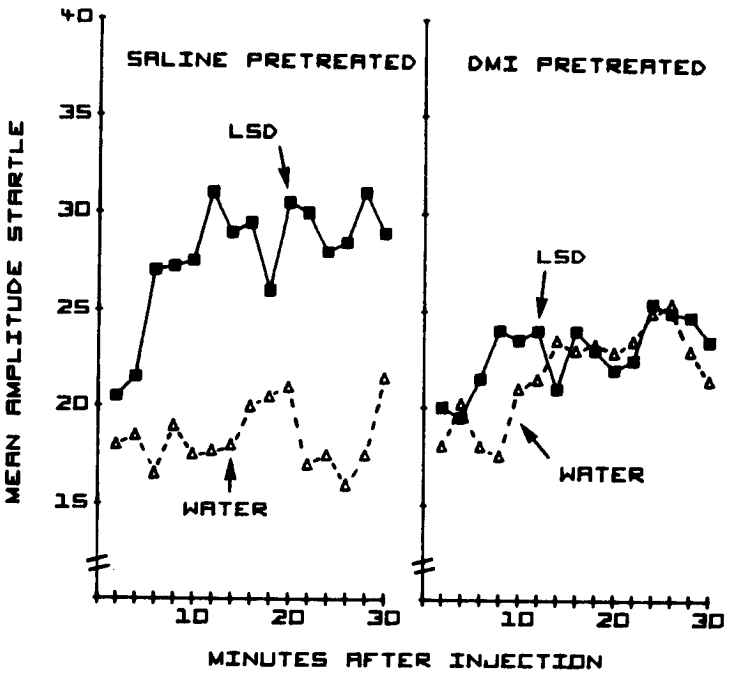

FIG. 2

Mean amplitude startle response over successive 2-min periods after injection with either LSD or water in rats pretreated 15 min earlier with either saline (left panel) or 5 mg/kg desipramine (right panel).

TABLE 2

Effects of CIMI and DMI Pretreatment on Brain Levels of ^{3}H-LSD

Treatment[1]	Whole brain ^{3}H-LSD (ng/g brain tissue & S.E.)
Saline	6.19 ± 0.17
CIMI (5 mg/kg)	8.09 ± 0.77[a]
DMI (5 mg/kg)	10.64 ± 0.36[b]

[1] Drugs (CIMI;DMI) or saline were administered 15 minutes prior to the administration of ^{3}H-LSD (30 µg/kg) and rats were sacrificed 15 minutes after receiving radioactive LSD, n = 8.

[a] $p < 0.05$ vs saline controls

[b] $p < 0.001$ vs saline controls

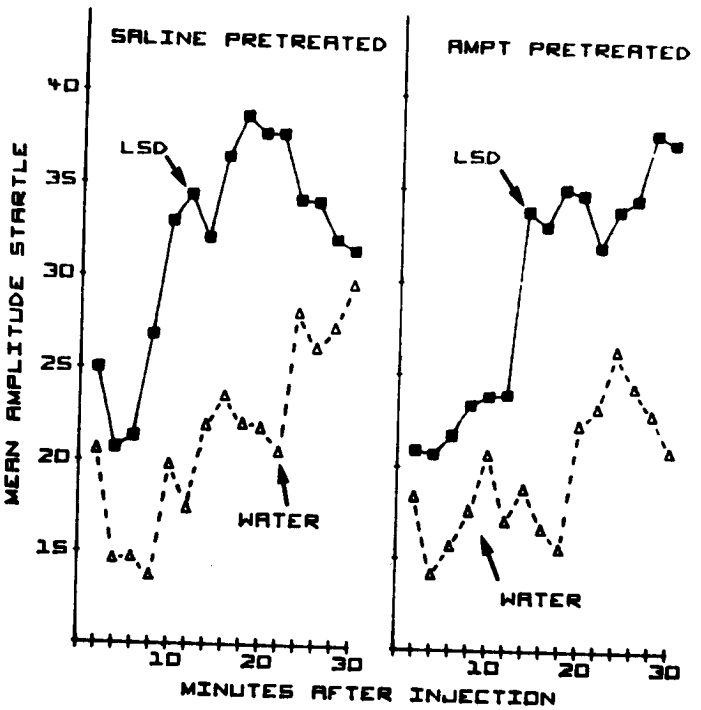

FIG. 3

Mean percent block of the LSD effect after pretreatment 15 min earlier with various doses of either desipramine or chlorimipramine.

Discussion

The major finding of the present study was that the tricyclic antidepressant drugs, chlorimipramine, imipramine, chlordesipramine, and desipramine were all about equally potent in blocking the usual excitatory effect of a low dose of LSD on the acoustic startle reflex. In contrast, the catecholamine synthesis inhibitor, AMPT, did not block the LSD effect. By themselves, DMI, C-DMI and IMI augmented startle about 20-30% at doses of 5 mg/kg, whereas CIMI did not have any significant effect on startle.

The observation that CIMI blocked the excitatory effect of LSD without altering startle by itself is consistent with the following proposed mechanisms of action of these drugs. LSD is believed to augment startle by inhibiting the firing rate of midbrain raphe neurons which in turn decreases release of 5-HT and disinhibits startle. CIMI also depresses the firing rate of raphe neurons but does so indirectly by blocking the reuptake of 5-HT. At this particular dose CIMI does not have any net effect on the release of 5-HT and hence would not be expected to alter startle. However, pretreatment with CIMI and the resultant depression of raphe cell firing should preempt the ability of LSD to alter startle if in fact LSD augments startle by inhibiting raphe cell firing. Since IMI also blocked the LSD effect on startle at a dose which depresses raphe cell firing (28), it is possible that both drugs block the LSD effect by a similar mechanism of action.

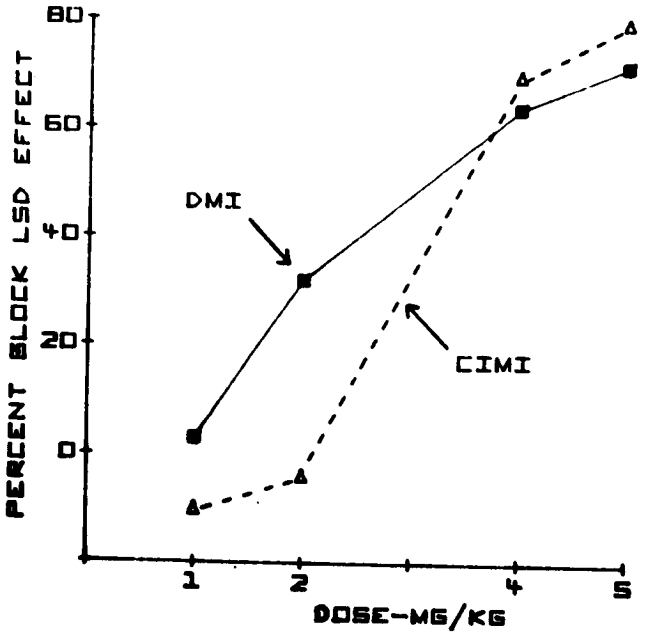

FIG. 4

Mean amplitude startle response over successive 2-min periods after injection with either LSD or water in rats pretreated 1 hr earlier with either saline (left panel) or 100 mg/kg α-methyl-p-tyrosine (right panel).

The finding that DMI and C-DMI also blocked the excitatory effect of LSD on startle cannot be explained by the above theoretical formulation. Compared to CIMI, these drugs are more potent in blocking reuptake of norepinephrine (NE) than 5-HT. For example, DMI is 100-300 times more potent in blocking NE than 5-HT reuptake and 40-100 times less potent in blocking 5-HT reuptake compared to CIMI (27). At the doses used in this study DMI does not depress raphe firing (28) but does have a potent depressive effect on the firing rate of the NE-containing neurons in the locus coeruleus (25). Yet, in spite of the fact that DMI is much less potent than CIMI in blocking 5-HT reuptake and depressing raphe cell firing, DMI was slightly more potent than CIMI in blocking the excitatory effect of LSD on startle.

In addition, all of the NE uptake blocking drugs by themselves had slight excitatory effects on startle. Yet when combined with LSD, which itself has an excitatory effect on startle, the two excitatory effects not only were not additive but instead seemed to cancel each other.

The ability of the tricyclic antidepressants to block the LSD effect on startle cannot be explained by a decrease in uptake or increase in metabolism of LSD since pretreatment with either CIMI or DMI did not decrease levels of ^{3}H-LSD in brain tissue. In fact, both DMI and CIMI significantly increased the amount of LSD measured in brain tissue. Since compounds of the imipramine

class are metabolized by hydroxylation in liver microsomal systems (22), and the metabolites of LSD are also most probably microsomal hydroxylation products (5,29,31), the increase in brain LSD suggests a competitive inhibition of metabolism. This effect appears similar to the well-studied interaction between imipramine and amphetamine in which an increase in brain levels of amphetamine observed after the administration of imipramine and similar agents has been shown to be due to an impairment of the hepatic metabolism of amphetamine (9,30,32).

The ability to block the LSD effect was somewhat specific to the antidepressant drugs, since AMPT, at a dose which essentially eliminates the excitatory effects of high doses of d- and l-amphetamine on startle, did not block the LSD effect. Thus AMPT differentiates between the excitatory effects of amphetamine and LSD on startle, as have specific behavioral startle tests (11, 14). However, different doses of AMPT as well as different injection-test intervals would have to be explored before definitive conclusions regarding the interaction between AMPT and LSD can be made, since it has been shown to alter excitatory sympathomimetic effects of LSD in other species (23).

The ability of antidepressant drugs to attenuate the excitatory effect of LSD is not limited to startle. Thus DMI and CIMI are about equally effective in blocking the excitatory effects of low doses of LSD on shock-elicited aggression in the rat (Sheard & Davis, unpublished observation). Perhaps similarly, the antidepressant monoamine oxidase inhibitors reduce the hallucinatory effects of LSD in humans (20,26).

At the present time it is not clear why the 5-HT and NE uptake blockers do not differ to a greater extent in their abilities to block the behavioral effects of LSD. The fact that they don't differ, however, may have important implications for the serotonin hypothesis of the action of hallucinogenic drugs. One possibility is that a NE synapse is located somewhere along the neural chain connecting the raphe-5-HT system to the final behavioral response. Thus even though LSD may act by inhibiting raphe neurons, concomitant depression of firing in NE neurons, caused by the NE uptake blocking drugs, may prevent the final behavioral expression of the LSD-induced reduction in 5-HT release. However, we have no evidence at this time that the ability of the tricyclic antidepressant drugs to block the excitatory effects of LSD is actually related to their effects on blocking reuptake and other mechanisms of action could be involved.

As outlined earlier, low doses of LSD are thought to increase startle by specifically depressing the firing rate of presynaptic raphe neurons. However, high enough doses of LSD and other hallucinogens can inhibit the firing rate of neurons postsynaptic to the raphe (3,21). Depending on the dose, therefore, hallucinogens either depress 5-HT transmission (low doses) or act as 5-HT agonists on neurons postsynaptic to the raphe (high doses). Consistent with these electrophysiological findings is the fact that hallucinogens have biphasic dose-response effects on acoustic startle, where low doses increase but high doses depress startle (10,15,19). It is conceivable that the tricyclic antidepressant drugs might in some way enhance the response of postsynaptic 5-HT receptors to LSD. If this were so, then a lower dose of LSD should now serve to depress startle and this might even be amplified by the higher levels of LSD in the brain after antidepressant drug pretreatment. Both effects would tend to move the biphasic dose-response startle curve to the left, so that now the depressant effect on startle caused by stimulation of postsynaptic 5-HT receptors could cancel the excitatory effect normally caused by inhibition of only the presynaptic 5-HT cells. This hypothesis leads to a number of specific preductions which are currently being tested.

Acknowledgements

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