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Effect of Neuroleptics and Tricyclic Antidepressants upon D-Amphetamine Discrimination

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Amphetamine Neuroleptics Drug-induced discrimination Antidepressants

THE "dopamine hypothesis" of schizophrenia states that a relative excess of dopaminergic neuronal activity may be an important feature of the mental dysfunction and this hypothesis is, at present, the predominant theory regarding a neurotransmitter defect in mental disease [3,32]. There exists a good correlation between the antipsychotic activity of neuroleptic drugs in humans and the effects of these drugs upon certain animal behavioral functions that are believed to be primarily under dopaminergic control. These behaviors, amphetamine-induced stereotypy and rotational behavior following unilateral destruction of the nigrostriatal dopamine neurons, have been extensively employed as preclinical screening procedures for potential neuroleptics, and, biochemically, it is possible to relate the behavioral changes with the drugs' dopaminergic receptor blocking action in the brain [22,35]. In contrast, there are few reliable animal models which both resemble depressive illness and are selectively sensitive to clinically effective antidepressant treatments. In a variety of animal behavioral paradigms that are thought to be associated with brain dopamine, antidepressant drugs have been observed to have effects that are opposite to the neuroleptics. For example, antidepressants potentiate the stimulant effects of L-dopa and apomorphine in rats that are blocked by neuroleptics, as well as antagonizing catalepsy induced by neuroleptics [8, 19, 29]. These opposing effects have, furthermore, been observed in man. Neuroleptics can produce Parkinsonian-like side effects which antidepressant drugs can partially alleviate [21] and anti-depressants have been observed to potentiate schizophrenic symptoms [15] which antipsychotic neuroleptics, by definition, help alleviate.

It has been shown that d-amphetamine is capable of controlling differential responding, i.e., drug-induced stimulus control, in rats after either intraperitoneal [13,28] or intravenous [1] administration. Additional evidence has led to the suggestion that this behavioral model, like stereotypy and rotational behavior, is dopaminergically mediated [11, 16, 27]. The purpose of the present study was to test the effects of various antipsychotic (neuroleptic) and tricyclic antidepressant drugs on rats' ability to discriminate d-amphetamine. If neuroleptics block dopamine receptors then the d-amphetamine-produced discriminative performance (cue) would be expected to be antagonized, whereas, if antidepressants block reuptake of dopamine into presynaptic neurons [9], these agents would be expected to potentiate the dopaminergically mediated discriminative behavior.

METHOD

Subjects

Sixteen male ARS/Sprague Dawley rats were individually

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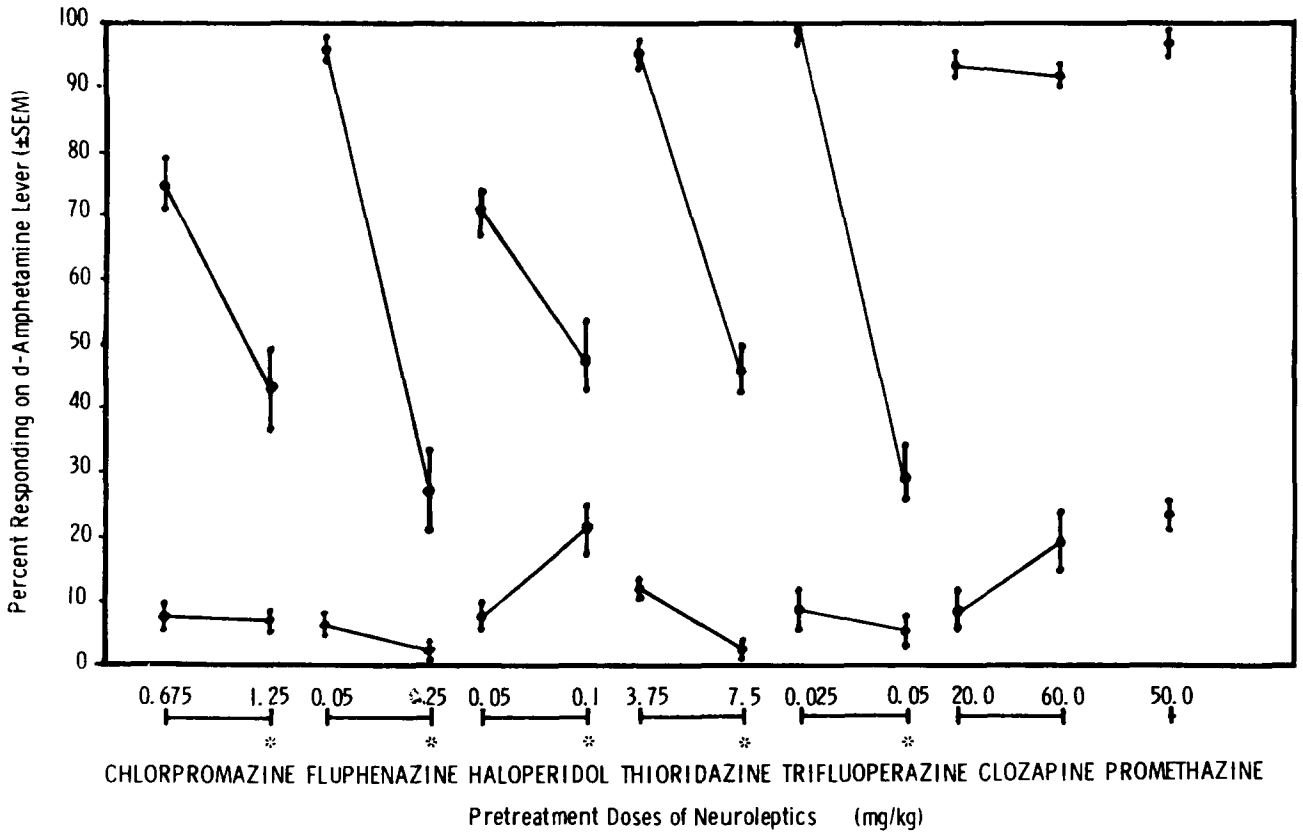

FIG. 1. Effect of pretreatment with neuroleptics upon d-amphetamine and saline discrimination. Percent responding on d-amphetamine lever after specific neuroleptic agent and saline (bottom figures) is expressed as the mean (±1 SEM) in 16 rats at each of two doses of neuroleptic administered on 2 occasions each. d-Amphetamine administered alone produced 95.4 ± 2.1 percent responding upon the d-amphetamine-correct lever, whereas, saline produced 3.4 ± 0.9 percent responding on the drug-appropriate lever. The asterisks below the doses of neuroleptics pertain to significance of difference (Wilcoxon matched-pairs signed-ranks test; $p<0.01$) between neuroleptic pretreatment and maintenance testing with 0.6 mg/kg d-amphetamine.

By session 36, with each treatment given on 18 occasions, the degree of drug-related responding after d-amphetamine reached a mean of 93%, whereas the degree of responding on the drug-appropriate lever after saline declined to less than 10%. Throughout the remainder of the experimentation, a high level of discriminated responding was evident with rats correctly depressing the d-amphetamine- or saline-correct lever on greater than 90% of total responses after either treatment. At no time during the course of this study was tolerance to the discriminative effects of d-amphetamine observed.

Figure 1 represents the results of administration of various neuroleptics prior to discriminative testing with d-amphetamine and saline. Although 3 doses of neuroleptics were used in some instances, the results only include the highest doses that had a significant effect on d-amphetamine discrimination without significantly affecting either saline discrimination or the total rates of responding when co-administered with d-amphetamine. The ED50 values for each effective neuroleptic were plotted according to the method of Litchfield and Wilcoxin [17] and were: trifluoperazine, 0.04 mg/kg (95% confidence range: 0.03–0.05 mg/kg); haloperidol, 0.09 mg/kg (0.05–0.18 mg/kg); fluphenazine, 0.16 mg/kg (0.11–0.23 mg/kg); chlorpromazine, 1.1 mg/kg (0.6–2.0 mg/kg) and thioridazine, 7.2 mg/kg (5.8–9.0 mg/kg). The administration of 20 and 60 mg/kg clozapine and 50 mg/kg promethazine had no significant effect on d-amphetamine discrimination

The slopes of the dose-response curves for the d-amphetamine discrimination antagonism by the effective neuroleptics were similar and the test for parallelism of slopes [17] indicated that no slope differed significantly from any other slope. The slope functions varied between 1.35 (trifluoperazine) and 2.71 (haloperidol). In contrast, all ED50 values for d-amphetamine cue antagonism differed significantly ($p<0.05$) from one another with the exception being the non-significant difference between the ED50 values for haloperidol (0.09 mg/kg) and fluphenazine (0.16 mg/kg). Thus, the potencies were: trifluoperazine>haloperidol= fluphenazine>>chlorpromazine>>thioridazine.

Figure 2 illustrates the effects of pretreatment with three tricyclic antidepressant agents upon the discriminative performance after a dose of d-amphetamine (0.05 mg/kg) which when administered alone produced a low degree of discriminative control (30.4%). When the tricyclics were administered prior to saline they produced mean percent responding similar to that seen after saline administration (9.9%), ranging from 12.6% with 20 mg/kg imipramine to 17.6% with 10 mg/kg nortryptiline. The administration of 20 mg/kg imip-

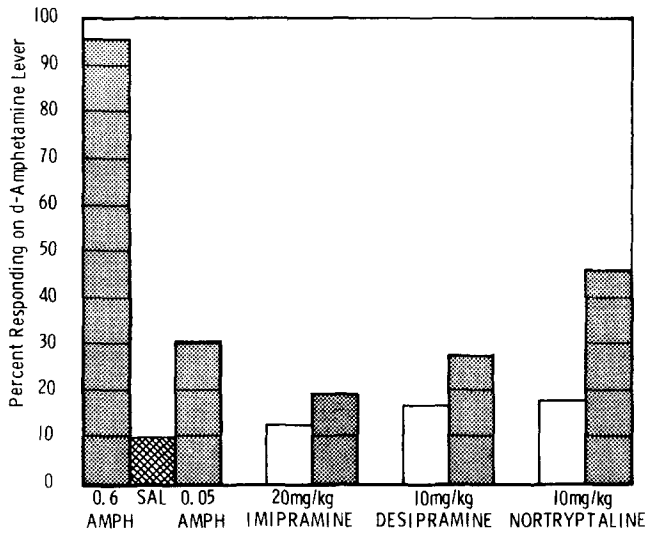

FIG. 2. Effect of pretreatment with tricyclic antidepressants upon discrimination of 0.05 mg/kg d-amphetamine and saline. The three bars on the left indicate mean percent responding on d-amphetamine lever after 0.6 mg/kg d-amphetamine, saline and 0.05 mg/kg d-amphetamine. Open bars indicate percent responding on d-amphetamine lever after administration of tricyclic antidepressant and saline, whereas stipled bars indicate mean percent responding after tricyclic antidepressant and 0.05 mg/kg d-amphetamine.

ramine or 10 mg/kg desipramine before 0.05 mg/kg d-amphetamine produced mean percent responding on the d-amphetamine correct lever that was less than that observed with 0.05 mg/kg d-amphetamine administered alone. The combination of 10 mg/kg nortryptiline and 0.05 d-amphetamine produced responses on the d-amphetamine lever that were slightly, but not significantly, higher than seen after 0.05 mg/kg d-amphetamine administration.

DISCUSSION

Several investigators have employed d-amphetamine as a discriminative stimulus in rats and they have demonstrated that when an appropiate antagonist is administered prior to the training dose of d-amphetamine, drug contingent discrimination is temporarily upset, i.e., the rats make the saline contingent choice. Effective antagonists have included the neuroleptics chlorpromaine [24], pimozide [13] and haloperidol [27]. There is convincing pharmacological and biochemical evidence that these agents are able to block dopamine receptor sites [12,33], and the finding that they are effective in antagonizing the discriminative stimulus properties of amphetamine corroborates the hypothesis that the discrimination of amphetamine by rats depends upon its ability to mimic dopaminergic agonist effects at postsynaptic sites [27].

The present study extends the previously cited reports by employing five effective neuroleptics and generating ED50 values for each. These values are similar to the ED50 values for neuroleptic antagonism of a higher amphetamine dose that produced stereotypic behavior in rats. Indeed, the values, for fluphenazine (0.1 mg/kg) chlorpromazine (1.1 mg/kg) and thioridazine (7.1 mg/kg) are identical [14]. Thus, it appears that d-amphetamine control of discriminative responding in rats, like amphetamine stereotyped behavior, is mediated by dopaminergic neurons as proposed by a previous investigation that employed dopaminergic agonists [25]. The parallelism of the dose-response curves for the neuroleptic antagonism indicates that they act on a common site and the simplest explanation is that the mechanism underlying the d-amphetamine-cue antagonism consists of blockade of dopamine receptors. A similar hypothesis has been offered to explain similar effects of neuroleptics upon the control of discriminative responding by apomorphine, a drug proposed to act as a direct dopamine agonist [7].

Pretreatment with a single large dose of promethazine, a phenothiazine derivative with antihistaminic, antiemetic and sedative properties but devoid of antipsychotic efficacy, and d-amphetamine produced d-amphetamine-appropriate responding. Likewise, administration of two doses of clozapine (20 and 60 mg/kg) did not antagonize the d-amphetamine cue. The antipsychotic efficacy of clozapine has been confirmed even though its mechanism of action appears to differ from that of other neuroleptics. Behaviorally, clozapine administered subcutaneously to rats provides no protection against amphetamine or apomorphine stereotypy [5]. Biochemically, clozapine increases dopamine concentration in the striatum in contrast to the decrease observed with other neuroleptics [6]. Clinically, clozapine possesses antipsychotic efficacy without the production of extrapyramidal side-effects [18,31]. Various investigators have explained these discrepancies by proposing that, even though clozapine may block dopamine receptors, the usual consequences of such blockade are masked by the pronounced anticholinergic and/or muscle relaxing properties of the drug [20, 33, 34]. This hypothesis is not universally accepted [4,6], and the explanations for the discrepancies cited must await further investigations [2]. The present investigation confirms that, in the behavioral paradigm used, clozapine produces effects different than those observed with the more "classical" neuroleptics.

In light of recent in vitro investigations that have shown that tricyclic antidepressants inhibit the uptake of dopamine into rat brain synaptosomal preparation [9,23], these agents were administered to rats prior to a dose of d-amphetamine that produced a low degree of discriminative performance. As shown in Fig. 2, pretreatment with the tricyclic antidepressants did not significantly increase d-amphetamine discrimination. In a recent report, Halaris and Feigenbaum [10] observed that similar doses of the antidepressants significantly potentiated amphetamine-induced stereotypic behavior in the rat and from this evidence they suggested that dopamine may play a significant role in the mode of action of tricyclic antidepressants. The present study does not appear to confirm this hypothesis.

The ability of agents that act as agonists on dopaminergic receptors to produce a d-amphetamine-like cue [25], and the present evidence indicating the ability of neuroleptic drugs that block dopamine receptors to antagonize response control by d-amphetamine, suggests that dopaminergic systems play a role in mediating the discriminative properties of d-amphetamine. Thus, d-amphetamine induced discriminative control in rats joins stereotypic and rotational behavior as animal behavioral methods for determining dopamine interactions in the central nervous system. This may allow for a better understanding of the mechanisms of action of drugs that are efficacious in psychosis and this sensitive, albeit indirect, method may shed some light upon the pathophysiological brain mechanisms involved.

REFERENCES

1. Ando, K. The discriminative control of operant behavior by intravenous administration of drugs in rats. *Psychopharmacologia* **45:** 47–50, 1975.
2. Ayd, F. J., Jr. Clozapine: A unique new neuroleptic. *Int. Drug Ther. Newsletter* **9:** 5–12, 1974.
3. Baldessarini, R. J. Schizophrenia. *New Engl. J., Med.* **297:** 987–995, 1977.
4. Bartholini, G., W. Haefeli, M. Jalfre, H. H. Keller and A. Pletscher. Effects of clozapine on cerebral catecholaminergic neuronal systems. *Br. J. Pharmac.* **46:** 736–740, 1972.
5. Bürki, H. R., E. Eichenberger, A. C. Sayers ad T. G. White. Clozapine and the dopamine hypothesis of schizophrenia, a critical appraisal. *Pharmakopsychiat.* **8:** 115–121, 1975.
6. Bürki, H. R., W. Ruch, H. Asper, M. Baggiolini and G. Stille. Effect of single and repeated administration of clozapine on the metabolism of dopamine and noradrenaline in the brain of the rat. *Eur. J. Pharmac.* **27:** 180–190, 1974.
7. Colpaert, F. C., J. E. M. F. Leysen, C. J. E. Niemegeers and P. A. J. Janssen. Blockade of apomorphine's discriminative stimulus properties: Relation to neuroleptic activity in neuropharmacological and biochemical assays. *Pharmac. Biochem. Bheav.* **5:** 671–679, 1976.
8. Everett, G. M. The DOPA response potentiation test and its use in screening for antidepressant drugs. In: *Antidepressant,* edited by S. Garattini and M. N. G. Dukes. Amsterdam: International Congress Series No. 122, Excerpta Medica Foundation, 1967, pp. 164–167.
9. Halaris, A. E., K. T. Belendink and D. X. Freedman. Antidepressant drugs affect dopamine uptake. *Biochem. Pharmac.* **24:** 1896–1898, 1975.
10. Halaris, A. E. and J. J. Feigenbaum. Potentiation of stereotyped behavior and locomotor activity with antidepressants: A dopaminergic effect? Neuroscience Abstracts of the Sixth Annual Meeting of the Soc. for Neurosciences. Toronto, Canada, p. 850, 1976.
11. Ho, B. T. and J. T. Huang. Role of dopamine in d-amphetamine-induced discriminative responding. *Pharmac. Biochem. Behav.* **3:** 1085–1092,1975.
12. Horn, A. S., J. T. Coyle and S. H. Snyder. Catecholamine uptake by synaptosomes from rat brain: Structure-activity relationships of drugs with differential effects on dopamine and norepinephrine neurons. *Molec. Pharmac.* **7:** 66–80, 1971.
13. Huang, J. T. and B. T. Ho. Discriminiative stimulus properties of d-amphetamine and related compounds in rats. *Pharmac. Biochem. Behav.* **2:** 669–673, 1974.
14. Janssen, P. A. J., C. J. E. Niemegeers and K. H. L. Schellekens. It is possible to predict the clinical effects of neuroleptic drugs (major tranquilizers) from animal data? I "neuroleptic activity spectra" for rats. *Arzneimittel-Forsch.* **15:** 104–117, 1965.
15. Klein, R. F. Behavioral effects of imipramine and phenothiazines: Implications for a psychiatric pathogenic theory and theory of drug action. In: *Recent Advances in Biological Psychiatry,* Vol. 7, edited by J. Wortis. New York: Plenum Press, 1965, pp. 273–287.
16. Kuhn, D. M., J. B. Appel and I. Greenberg. An analysis of some discriminable properties of *d*-amphetamine. *Psychopharmacologia* **39:** 57–66, 1974.
17. Litchfield, J. T. and F. Wilcoxin. A simplified method of evaluating dose-effect experiments. *J. Pharmac. exp. Ther.* **96:** 99–113, 1949.
18. Matz, R., W. Rick, H. Thompson and S. Gershon. Clozapine: Potential antipsychotic agent without extrapyramidal manifestations. *Curr. Ther. Res.* **16:** 687–695, 1974.
19. McGrath, W. R. and H. J. Kettler. Potentiation of the antireserpine effects of dihydroxyphenylalanine by antidepressants and stimulants. *Nature* **199:** 917–918, 1963.
20. Miller, R. J. and C. R. Hiley. Antimuscarinic properties of neuroleptics and drug-induced parkinsonism. *Nature* **248:** 596–597, 1974.
21. Morselli, P. L., M. Rizzo, M. Zaccala and S. Garattini. Haloperidol-desipramine interaction in mice, rats, and man. *Chem.-Biol. Interactions* **2:** 160–164, 1970.
22. Randrup, A. Role of brain dopamine in the antipsychotic effect of neuroleptics: Evidence from studies of amphetamine-neuroleptic interaction. In: *The Neuroleptics,* edited by D. P. Bobon, P. A. J. Janssen and J. Bobon. Basel: S. Karger, 1970, pp. 60–65.
23. Randrup, A. and C. Braestrup. Uptake inhibition of biogenic amines by newer antidepressant drugs: Relevance to the dopamine hypotheses of depression. *Psychopharmacology* **53:** 309–314, 1977.
24. Roffman, M. and H. Lal. Role of brain amines in learning associated with "amphetamine-state". *Psychopharmacologia* **25:** 195–204, 1972.
25. Schechter, M. D. Amphetamine discrimination as a test for anti-parkinsonism drugs. *Eur. J. Pharmac.* **44:** 51–56, 1977.
26. Schechter, M. D. Stimulus properties of *d*-amphetamine as compared to *l*-amphetamine. *Eur. J. Pharmac.* **47:** 461–464, 1978.
27. Schechter, M. D. and P. G. Cook. Dopaminergic mediation of the interoceptive cue produced by *d*-amphetamine in rats. *Psychopharmacologia* **42:** 185–193, 1975.
28. Schechter, M. D. and J. A. Rosecrans. D-amphetamine as a discriminative stimulus: Drugs with similar stimulus properties. *Eur. J. Pharmac.* **21:** 211–216, 1973.
29. Scheel-Krüger, J. Central effect of anticholinergic drugs measured by the apomorphine gnawing test in mice. *Acta Pharmac.* **28:** 1–16, 1970.
30. Siegel, S. Nonparametric Statistics for the Behavioral Sciences. New York: McGraw Hill Book, Co., 1956.
31. Simpson, G. M. and E. Varga. Clozapine: New antipsychotic agent. *Curr. Ther. Res.* **16:** 679–686, 1974.
32. Snyder, S. H. The dopamine hypothesis of schizophrenia: Focus on the dopamine receptor. *Am. J. Psychiat.* **133:** 197–202, 1976.
33. Snyder, S. H., D. Greenberg and H. I. Yamamura. Antischizophrenic drugs and brain cholinergic receptors. *Arch. Gen. Psychiat.* **31:** 58–61, 1974.
34. Stadler, H., K. G. Lloyd and G. Bartholini. Dopaminergic inhibition of striatal cholinergic neurons. Synergistic blocking action of α-butyrolactone and neuroletpic drugs. *Naunyn-Schmiedberg's Arch. Pharmak.* **283:** 129–134, 1974.
35. Ungerstedt, U. Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behavior. *Acta Physiol. Scand. suppl.* **367:** 49–67, 1971.