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Atypical Neuroleptics Clozapine and Thioridazine Enhance Amphetamine-Induced Stereotypy

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ROBERTSON, A. AND C. MACDONALD. *Atypical neuroleptics clozapine and thioridazine enhance amphetamine-induced stereotypy*. PHARMACOL BIOCHEM BEHAV **21**(1) 97–101, 1984.—The effects of the atypical neuroleptics clozapine and thioridazine and the typical neuroleptic pimozide on amphetamine-induced behavior were examined. Pimozide, as expected, blocked both amphetamine-induced locomotion and stereotypy. Thioridazine and clozapine antagonized the increases in locomotion produced by amphetamine, but produced increases in amphetamine-induced stereotypy and lowered the threshold dose for stereotypy. It is suggested that the increased stereotypy might partly account for the decreased locomotion, and that this might be a primary effect of these atypical neuroleptics. The data would also suggest that the use of amphetamine-induced stereotypy as a model for psychosis is inappropriate, as clozapine and thioridazine, which enhance stereotypy, are antipsychotic.

Typical and atypical neuroleptics Locomotion Stereotypy Clozapine Amphetamine Pimozide Thioridazine

ANTAGONISM of apomorphine- or amphetamine-induced behavior has been widely used as a pharmacological measure of the activity of dopamine antagonists, or neuroleptics. Typical or classical neuroleptics (those highly associated with a variety of extrapyramidal side effects in the laboratory and clinic) block a wide range of amphetamine-induced behaviors, including locomotion and stereotypy. In contrast, atypical neuroleptics (those associated with a much lower incidence of extrapyramidal side effects) have much more variable effects on amphetamine-induced behavior. It is widely reported that atypical neuroleptics such as clozapine and thioridazine are able to block amphetamine- or apomorphine-induced facilitation of locomotion, but are relatively ineffective in blocking amphetamine- or apomorphine-induced stereotypy [9, 14, 16, 20, 26]. There are, however, contradictory reports [3, 4, 18, 22] including reports that clozapine can increase stereotypy [1, 7, 15]. These contradictory data call into question the reliability or the usefulness of the amphetamine-apomorphine model for assessing and differentiating neuroleptic drugs. We thought, however, that the reports of increased stereotypy following clozapine administration seemed interesting and might account for some of the other reported results. Many experiments are designed so that administration of the agonist maximizes the measured incidence of the behavior under study (usually stereotypy or locomotion). Therefore decreases in that behavior are easy to measure but increases are correspondingly difficult or impossible due to the ceiling effect. If atypical neuroleptics have the effect of increasing stereotypy, this would not be observed whenever asymptotic doses of the agonist are used.

The objective of the present experiment was to test systematically the hypothesis that the atypical neuroleptics clozapine and thioridazine can increase stereotypy. This seemed important not only because antagonism of amphetamine-induced behaviors is widely used to measure neuroleptic activity but because it has been used as a model to study the neural basis of neuroleptic effects (e.g., [4]). Moreover, the production of stereotypy following amphetamine administration has been used as a model for idiopathic or drug-induced paranoid schizophrenia [8, 13, 19, 21]. If atypical neuroleptics, which combat psychosis, produce stereotypy, then that model would not seem useful.

We measured the effectiveness of clozapine and thioridazine in altering stereotyped behaviors as well as locomotion produced by a range of doses of amphetamine, and contrasted these effects with those produced by administration of pimozide, a typical neuroleptic associated with a very potent antagonism of amphetamine-induced locomotor and stereotyped behaviors in the rat [10,20] and associated with some degree of extrapyramidal side effects in the clinic [23].

METHOD

Subjects

Seventy-five male Long-Evans rats (Charles River Canada Inc., St. Constant, Quebec), weighing from 250–275

g at the start of the experiment, were used. They were housed in groups of 3 per cage, with food and water available ad lib on a 12:12 hr dark:light schedule. All testing was carried out during the light period.

Activity Measures

During each test session, a rat was placed in a rectangular box (45×45×30 cm) with three wooden walls, one Plexiglas wall, and a grid floor. Three such boxes were used, with each rat assigned to one box for the duration of the experiment. Each box was equipped with four sets of photocell beams, two beams equally spaced on each wall about 1.5 cm above the floor. Breaking of each beam was automatically recorded throughout all test sessions on mechanical counters.

Visual observations were taken during six three-minute intervals, at 5, 15, 25, 35, 45 and 55 min of each 60 min test session. During these intervals, a metronome clicked every nine sec. At each click, the behavior of the rat was recorded. In all instances, it was then classified into one of the categories listed in Table 1.

Therefore there were 120 observations for each 60 min test session which were divided accordingly amongst the possible categories.

Drugs

Pimozide and clozapine were dissolved in 1% lactic acid. Pimozide was administered SC 3.5 hours before testing, in doses of 0, 0.125, 0.250 and 0.500 mg/kg. Clozapine was administered SC 0.5 hr before testing in doses of 0, 5.0 and 10.0 mg/kg. Thioridazine HCL, dissolved in sterile isotonic saline, was administered SC 0.5 hr before testing in doses of 0, 2.5, 5.0 and 10.0 mg/kg. D-amphetamine sulphate was dissolved in sterile isotonic saline and administered IP at the beginning of the test session in doses of 0, 1.0, 2.5 and 5.0 mg/kg. All were injected in a volume of 1.0 ml/kg.

Procedure

Rats were first randomly divided into the three drug groups: pimozide, clozapine and thioridazine. Within each group, rats were then randomly assigned, six–seven rats per group, to each dosage condition. Therefore there were four thioridazine groups (0, 2.5, 5.0 and 10.0 mg/kg), four pimozide groups (0, 0.125, 0.250 and 0.500 mg/kg) and three clozapine groups (0, 5 and 10 mg/kg).

On the first three days of the experiment, animals were placed in the test boxes for 25 min habituation periods. The following day, animals remained in the boxes for 60 min, during which baseline scores of behavior were recorded. Drug testing then began. For each drug test, the rat would receive the appropriate dose of neuroleptic or its vehicle, and then, in ascending order, 0.9% saline, 1.0 mg/kg amphetamine, 2.5 mg/kg amphetamine and finally 5.0 mg/kg amphetamine. Rats were allowed at least three days between each drug test, and baseline scores were taken regularly to ensure that there were no detectable post-drug alterations in normal responding.

RESULTS

Types of Behavior Measured

Behaviors which were observed to occur in a stereotyped fashion (after the definition of Fog ([6], p. 14)—"decreased variation in behavior, continuous repetition of behavior patterns or items") included sniffing down, nose-poking, and repetitive head movements. Behaviors which were observed to occur, but not in a stereotyped fashion, included locomotion, rearing, grooming, sniffing with the head up, standing still, and lying or resting prone.

TABLE 1
BEHAVIORAL CATEGORIES

Locomotion	Rearing
Grooming	Sniffing with Head-Up
Standing Still	Lying or Resting Prone
Gnawing	Repetitive Locomotion
Sniffing Down	Nose-Poking
Foot Shuffling	Repetitive Head Movements
Licking	Other

An average of 97% of behaviors occurring in a stereotyped fashion, across all conditions, were classified as either sniffing down (which meant that the snout was not raised more than about 1 cm above ground level) or repetitive head movements. Of the two of these, sniffing down occurred the majority of times. Under all conditions, as the absolute amount of stereotypy increased, the relative amount of sniffing down would decrease while the relative amount of repetitive head movements would increase.

Data Analysis

Of the 120 observations recorded for each rat for each one-hour period, only those for locomotion and stereotyped behaviors were used in the data analysis. The cumulative one-hour observations for all stereotyped behaviors were combined to form one dependent variable, and the total number of observations of locomotion for the one-hour period formed the second dependent variable.

Photocell beam counts were recorded throughout all test sessions but were not used in the analysis as a measure of locomotion, because photocell beam counts under conditions where a lot of stereotypy was occurring were often clearly augmented by this stereotypy. Using only the visual observation data ensured that locomotion and stereotyped behaviors were rated on a mutually exclusive basis. The photocell beam data were used, however, to assess the consistency of the data from the visual observations. For 18 subjects, counts from photocell beams were correlated with visual locomotor counts under baseline conditions and following administration of 1 mg/kg amphetamine, as these two conditions were not associated with stereotypy. The Pearson Product Moment correlation coefficient was +.863 ($p<0.01$). As a second method of judging the visual rating scheme, the consistency of observations over each of the three vehicle control groups run were compared. These data can be seen in Figs. 1, 2 and 3. Finally, scores obtained with the same rating system used by a different observer, with different rats, in different boxes were compared for a dose of 2.5 mg/kg amphetamine. Scores for this second group were not appreciably different, averaging 3% higher for stereotypy ratings and 15% higher for locomotor ratings.

Data were analyzed separately for each drug, using two-way analyses of variance (neuroleptic dose × amphetamine dose) with repeated measures on the latter factor. ANOVA

TABLE 2
SUMMARY OF ANALYSES OF VARIANCE

Analysis	Source	DF	Locomotion F	Locomotion p	Stereotypy F	Stereotypy p
Pimozide	Pimozide	3,23	56.50	<0.001	36.88	<0.001
	Amphetamine	3,69	33.93	<0.001	103.04	<0.001
	Pim × Amph	9,69	11.38	<0.001	21.13	<0.001
Clozapine	Clozapine	2,18	4.56	0.025	1.83	0.189
	Amphetamine	3,54	9.76	<0.001	171.25	<0.001
	Cloz × Amph	6,54	2.92	0.015	2.71	0.023
Thioridazine	Thioridazine	3,23	8.92	<0.001	5.30	0.006
	Amphetamine	3,69	89.36	<0.001	301.55	<0.001
	Thior × Amph	9,69	3.34	0.002	2.09	0.043

summary tables are shown in Table 2. This was followed by *a posteriori* multiple comparisons amongst means using Tukey's procedure [11]. The level of significance in all cases was $p<0.05$.

Effects of Pimozide

As seen in Fig. 1, the 1 mg/kg and 2.5 mg/kg doses of amphetamine alone significantly increased locomotion; this increase was significantly attenuated by pimozide in a dose-dependent fashion. The 5 mg/kg dose of amphetamine did not produce significant changes in locomotion.

Stereotypy was significantly enhanced only by the 5 mg/kg dose of amphetamine. Pimozide produced a dose-dependent decrease of this stereotypy.

Effects of Clozapine

As was the case in the pimozide groups for the animals that received amphetamine alone, rats that received amphetamine but no clozapine showed significant increases in locomotion at the 1 and 2.5 mg/kg doses, but not at the 5.0 mg/kg dose (Fig. 2). The addition of 5 mg/kg or 10 mg/kg clozapine antagonized these increases for both the 1 and 2.5 mg/kg doses of amphetamine.

Although administration of 2.5 mg/kg amphetamine did not produce significant stereotypy relative to saline or 1.0 mg/kg amphetamine, the 2.5 mg/kg dose of amphetamine combined with 5.0 mg/kg of clozapine produced a significant amount of stereotypy relative to the lower doses of amphetamine and clozapine. The addition of 10 mg/kg clozapine produced a further increase—stereotypy scores with this combination were significantly higher than for rats that received 2.5 mg/kg amphetamine alone. The 5.0 mg/kg dose of amphetamine alone did produce a significant amount of stereotypy. The addition of clozapine produced slight (but not significant) increases.

Effects of Thioridazine

As seen in Fig. 3, 1 mg/kg amphetamine alone again produced a significant increase in locomotor scores, which was not significantly altered by thioridazine administration. However all three doses of thioridazine did significantly decrease the locomotor scores produced by 2.5 mg/kg amphetamine. The 5 mg/kg dose of amphetamine alone did not produce significant locomotion.

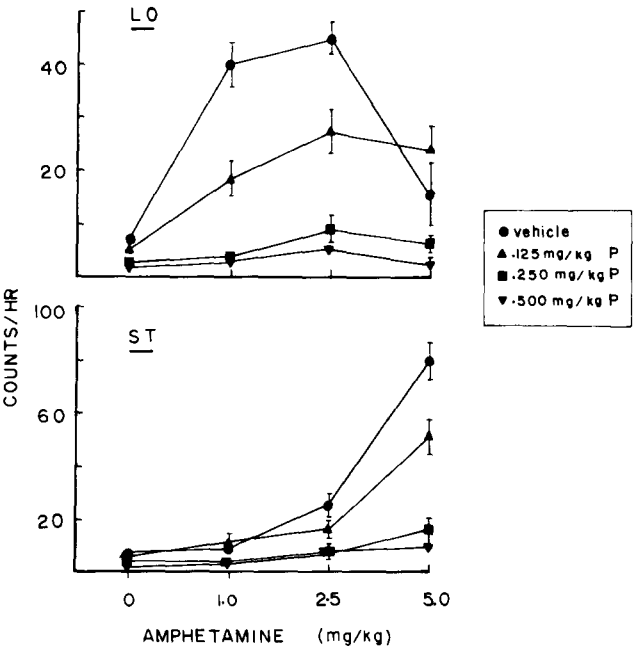

FIG. 1. Effects of pimozide (P) on locomotion (LO) and stereotypy (ST) produced by amphetamine. Vertical bars represent standard errors.

The 2.5 mg/kg amphetamine alone did not produce significant stereotypy relative to saline or 1.0 mg/kg amphetamine; the addition of 2.5, 5.0 or 10.0 mg/kg of thioridazine increased stereotypy so that it was significantly higher than control scores. Moreover, the combination of 10 mg/kg thioridazine with 2.5 mg/kg amphetamine produced significantly more stereotypy than 2.5 mg/kg amphetamine alone. The 5.0 mg/kg dose of amphetamine produced a significant increase in stereotypy. This was significantly enhanced only by the 10 mg/kg dose of thioridazine.

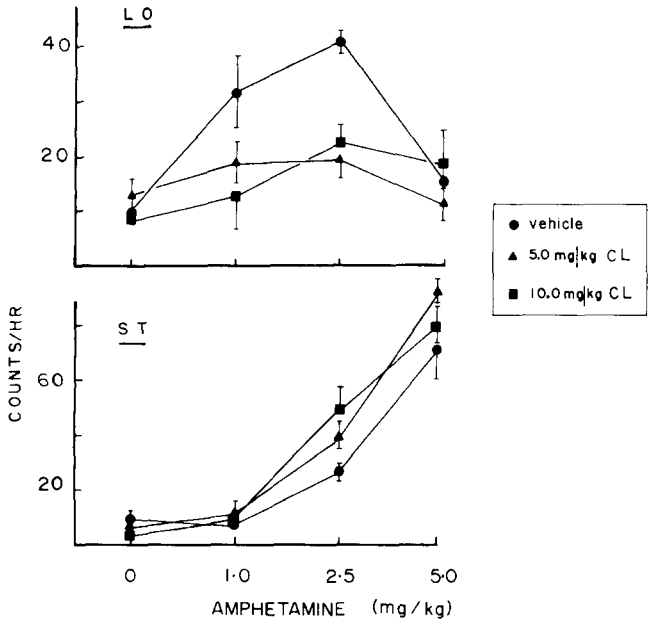

FIG. 2. Effects of clozapine (CL) on locomotion (LO) and stereotypy (ST) produced by amphetamine. Vertical bars represent standard errors.

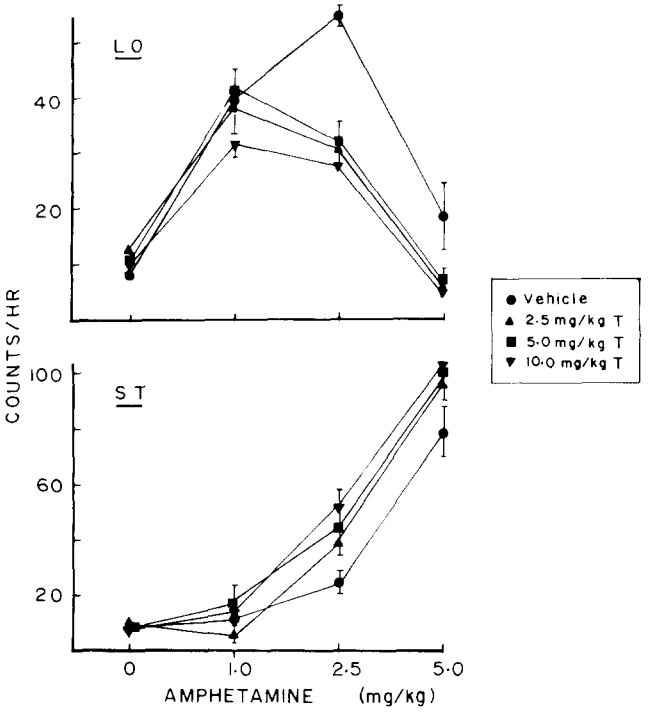

FIG. 3. Effects of thioridazine (T) on locomotion (LO) and stereotypy (ST) produced by amphetamine. Vertical bars represent standard errors.

DISCUSSION

Clozapine and thioridazine, known as atypical neuroleptics, enhanced amphetamine-induced stereotypy. The effectiveness of clozapine and thioridazine in so doing was most clearly apparent at a submaximal dose of amphetamine (2.5 mg/kg). At the highest dose of amphetamine, where stereotypy counts were approaching the ceiling (120 counts/hr), clozapine and thioridazine did not, for the most part, cause significant increases. Thus the effects of this nearly maximal dose of amphetamine would be entirely consistent with the majority of studies cited in the Introduction in finding little or no alteration of amphetamine-induced stereotypy following administration of atypical neuroleptics. It is only when the dose-response curve for amphetamine-induced stereotypy is observed that the facilitatory effects of clozapine and thioridazine become apparent. We do not observe stereotypy following administration of either of these neuroleptics alone. (In fact, we have noted in pilot experiments that rats appear progressively more and more disabled by these drugs in doses above 10 mg/kg and up to 50 mg/kg.) Moreover, the increased stereotypy scores observed when the atypical neuroleptic was added seemed to represent an enhancement of the same stereotyped behavior (repetitive head movements) rather than the production of a different type of stereotyped behavior.

In contrast to the clear dissociation of effects on amphetamine-induced stereotypy and locomotion produced by clozapine and thioridazine, pimozide produced the standard profile of typical neuroleptics: a clear dose-dependent reduction in both amphetamine-induced locomotion and stereotypy.

These data could be interpreted to mean that a primary effect of atypical neuroleptics (at least clozapine and thioridazine), in direct contrast to typical neuroleptics, is to enhance stereotyped behavior produced by amphetamine. Such an effect could, in part, account for the ability of atypical neuroleptics to block locomotion as, in all cases where stereotypy was enhanced significantly (usually more than 40 counts/hour), locomotor activity was reduced relative to the appropriate amphetamine comparison group. This would be expected by virtue of the definition of stereotypy. At the same time, it should be noted that, while such an analysis could account for all the observed instances of antagonism of amphetamine-induced locomotion in the thioridazine groups, clozapine did produce decreases in locomotion produced by 1 mg/kg amphetamine, but did not increase stereotypy counts. Therefore, although the effects of atypical and typical neuroleptics on amphetamine-induced locomotion are not clearly dissociable, their effects on amphetamine-induced stereotypy are, and appear potentially suitable as an explanatory model for studying the neural basis for the different degrees of extrapyramidal side effects produced by these drugs. These data would not, however, appear to support the use of amphetamine-induced stereotypy as a model for schizophrenia. Moreover, if stereotypy is used as a measure of dopaminergic activity in the brain (e.g., [6]), then these results would be incompatible with the idea that the therapeutic effects of antipsychotic drugs may be directly related to dopamine receptor blockade. A similar contention has already been made by Crow and Gillbe [5] based on the relative potencies of chlorpromazine and thioridazine in antagonizing amphetamine-induced turning in rats with unilateral electrolytic lesions of the substantia nigra.

It is unclear at the present time what properties of clozapine and thioridazine could account for the enhance-

ment of amphetamine-induced stereotypy. There are, however, reports in the literature [2,12] demonstrating that anticholinergic drugs increase and cholinergic drugs decrease amphetamine-induced stereotypy. Both clozapine and thioridazine have been reported to have anticholinergic effects [17,24] and this could account for the enhanced stereotypy. In this regard, it would be interesting to test sulpiride, an atypical neuroleptic, which has been reported to lack anticholinergic properties [25].

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