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Effects of Phencyclidine and Methylphenidate on *d*-Amphetamine-Induced Behaviors in Reserpine Pretreated Rats

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FESSLER, R. G., R. D. STURGEON AND H. Y. MELTZER. *Effects of phencyclidine and methylphenidate on d-amphetamine-induced behaviors in reserpine pretreated rats.* PHARMAC. BIOCHEM. BEHAV. 13(6) 835–842, 1980.—The effects and interactions of phencyclidine (PCP), methylphenidate and *d*-amphetamine on locomotor activity, stereotyped behavior and ataxia in reserpine- and vehicle-pretreated rats were examined. The behaviors of rats receiving PCP alone or in combination with other drugs were quantified along three dimensions (locomotor activity, stereotyped behavior, and ataxia) on scales developed in this laboratory. The behaviors of groups receiving methylphenidate and/or *d*-amphetamine in treatment combinations other than those including PCP were quantified using a well known *d*-amphetamine behavioral rating scale. PCP, methylphenidate and *d*-amphetamine each induced significant increases in locomotor activity and stereotyped behavior when administered alone. Reserpine was found to antagonize PCP-induced locomotor activity and stereotyped behavior, and methylphenidate-induced stereotyped behavior at a dose which either potentiated or had no significant effect upon *d*-amphetamine-induced behavior (depending upon the scale used). Reserpine also potentiated PCP-induced ataxia. Whereas PCP potentiated the locomotor activity induced by *d*-amphetamine in both reserpine- and vehicle-pretreated subjects, methylphenidate marginally antagonized *d*-amphetamine-induced stereotypy in reserpine-pretreated subjects. PCP-induced ataxia in reserpine pretreated subjects appeared moderately reduced in subjects also receiving *d*-amphetamine. In general, the behavioral effects of PCP appear to be more similar to those of methylphenidate than to those of *d*-amphetamine, but differences are also found between PCP and methylphenidate. The results are discussed in relation to a behavioral model recently proposed as a method for differentiating indirect dopamine agonists on the basis of their neurochemical mechanisms of action.

Phencyclidine *d*-Amphetamine Methylphenidate Reserpine Locomotor activity Stereotypy Ataxia Rat

PHENCYCLIDINE (PCP), a general anesthetic agent developed in the late 1960's, has been reported to induce psychotomimetic reactions in man [17,18]. The locomotor activity and some aspects of the stereotyped behavior produced by PCP appear similar to the effect of *d*-amphetamine and methylphenidate [3, 8, 9] suggesting the possibility of similar neurochemical effects for these drugs. *d*-Amphetamine and methylphenidate are believed to produce their behavioral effects mainly through presynaptic actions on catecholaminergic neurons. There are, however, important differences between their specific presynaptic effects. Whereas *d*-amphetamine is believed to act mainly by stimulating the release of newly synthesized dopamine (DA) from an alpha-methylparatyrosine (AMPT)-sensitive pool and by blocking DA uptake [2, 7, 29, 34], methylphenidate appears to act mainly via release of DA stored in reserpine-sensitive granules [4, 6, 25] and through blockade of DA uptake [7]. These differences in proposed mechanisms of action are probably quantitative rather than absolute [25].

Data from behavioral and neuropharmacological experiments are consistent with the hypothesis that many of the effects of PCP are also mediated by presynaptic effects on striatal DA neurons. In rats with unilateral 6-hydroxydopamine-induced lesions of the substantia nigra, PCP, like *d*-amphetamine [31] and methylphenidate [33], produces ipsilateral rotation [8], an action consistent with an indirect DA effect rather than a direct agonist action [31]. It was also reported that PCP decreased the accumulation of ^{14}C-DA and ^{14}C-norepinephrine (NE) and increased the levels of 0-methylated metabolites of these catecholamines formed from ^{14}C-tyrosine by brain [14], suggesting increased release or blockade of catecholamine uptake as the mechanism of action for PCP. An effect on tyrosine hydroxylase activity could also account for these data. Concordant with these findings, PCP significantly inhibits reuptake of NE and DA in synaptosomal homogenates prepared from whole brain or caudate, respectively [12, 26, 27]. Therefore, substantial evidence suggests a presynaptic mechanism of action for

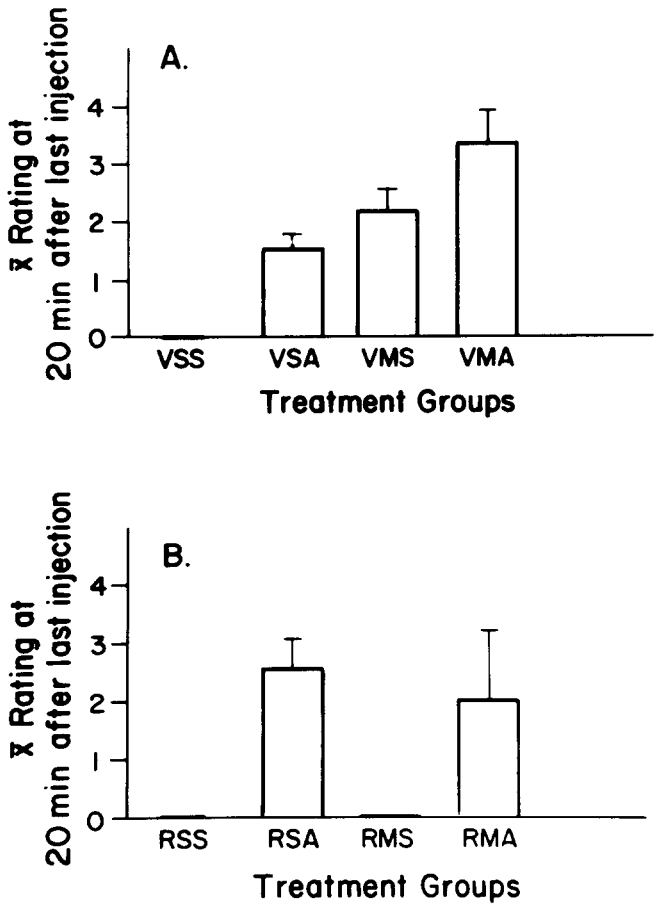

FIG. 1. Behavioral stereotypy of subjects receiving saline (S), *d*-amphetamine (A), methylphenidate (M), or combinations of these treatments following reserpine (R) or vehicle (V) pretreatment using the amphetamine stereotypy scale [5]. Bars indicate standard deviation. See text for time parameters.

PCP, but it is still unknown whether this effect is exerted primarily through the action on newly synthesized DA (an amphetamine-like effect), primarily through granular stored DA (a methylphenidate-like effect), through the combination of both, or through some unique process.

Recently, a new approach has been reported to successfully discriminate between the effects of *d*-amphetamine and methylphenidate. Both *d*-amphetamine and methylphenidate inhibit the accumulation of ^{3}H-DA into rat striatal homogenates and mouse forebrain homogenates [20, 21, 23, 24]. However, reserpine potentiates the inhibitory effect of *d*-amphetamine on DA accumulation but not that of methylphenidate [20,23]. The significance of this difference becomes apparent through examination of the factors proposed to affect biogenic amine accumulation of neural tissue. First, the inhibitory effect of reserpine on DA accumulation is believed to be a direct consequence of reduced vesicular storage capacity. Since it has been proposed that carrier-mediated release of DA increases under conditions in which the intraneuronal DA concentration exceeds the storage capacity of the synaptic vesicles [19], it is conceivable that reserpine-induced reduction of DA accumulation results from increased outward-directed membrane transport of the larger nonvesicular bound DA pool. This theoretically affects *d*-amphetamine and methylphenidate differently because of their different proposed presynaptic effects. Because *d*-amphetamine is believed to increase the release of extra-vesicular DA, the increased pool of extravesicular DA resulting from reserpine administration facilitates outward-directed transport and therefore potentiates the inhibition of DA accumulation. However, the major effect of methylphenidate is believed to be on vesicular-stored DA and blockade of DA uptake [4, 6, 7, 25]. Presumably, the common effects of reserpine and methylphenidate on vesicular DA would be redundant, and therefore potentiation of inhibited accumulation via an extravesicular DA pool would not be apparent. The lack of reserpine-induced potentiation of the antagonism of DA accumulation resulting from methylphenidate-induced "uptake blockade" is not as clear, but could be explained if the membrane carrier mediating uptake is the same carrier as that mediating release [10]. It has also been reported that reserpine does not potentiate the antagonism of DA accumulation induced by cocaine [24], a drug believed to be a relatively pure uptake blocker.

Based on the above data and hypotheses, and the reports that uptake inhibitors antagonize the release of DA induced by indirect agonists [21, 24, 30] the hypothesis was suggested that if methylphenidate was indeed a DA uptake blocker *in vivo*, it should antagonize behaviors resulting from *d*-amphetamine-induced DA release in reserpinized rats. In these rats, the effects of methylphenidate itself on behavior has been minimized because of the loss of stored DA. Consistent with this hypothesis, methylphenidate has been reported to inhibit *d*-amphetamine-induced stereotyped behavior in reserpine-pretreated rats [22,23].

In the following experiment, we have utilized the same paradigm [22,23] to obtain further information concerning whether the mechanism by which PCP exerts its behavioral effects has more similarity to that of methylphenidate or to *d*-amphetamine. Substantial similarity was observed between the effects of PCP and methylphenidate; however, our data suggest that PCP also must have some neurochemical actions which differentiate it from methylphenidate.

METHOD

Seventy-two male Sprague-Dawley rats (180–250 g), group housed in a temperature controlled environment prior to the experiment, served as the subjects for this experiment. Lights were on from 5 a.m. to 7 p.m. daily and Purina Lab Chow and water were available ad lib. On 6 consecutive days 12 randomly selected rats were moved to the experimental room and placed, 3 per cage, into 4 clear plastic cages (inside dimensions: 42×21×19 cm). The bottom of the cage was covered with wood chips. All rats within a cage received the same treatment combination; no subjects received more than one treatment combination. Assignment of the 3 rats within a cage to one of 12 treatment combinations was done randomly. Each of the 12 treatment groups had a total of 6 subjects. All subjects were allowed at least 1 hour to habituate to their environment.

Drug treatment combinations for the 12 groups of rats are presented in Figs. 1a and b and 2a and b. Because the behaviors elicited by *d*-amphetamine and methylphenidate are qualitatively different from those induced by PCP, quantitative ratings for the data reported in Figs. 1 and 2 were

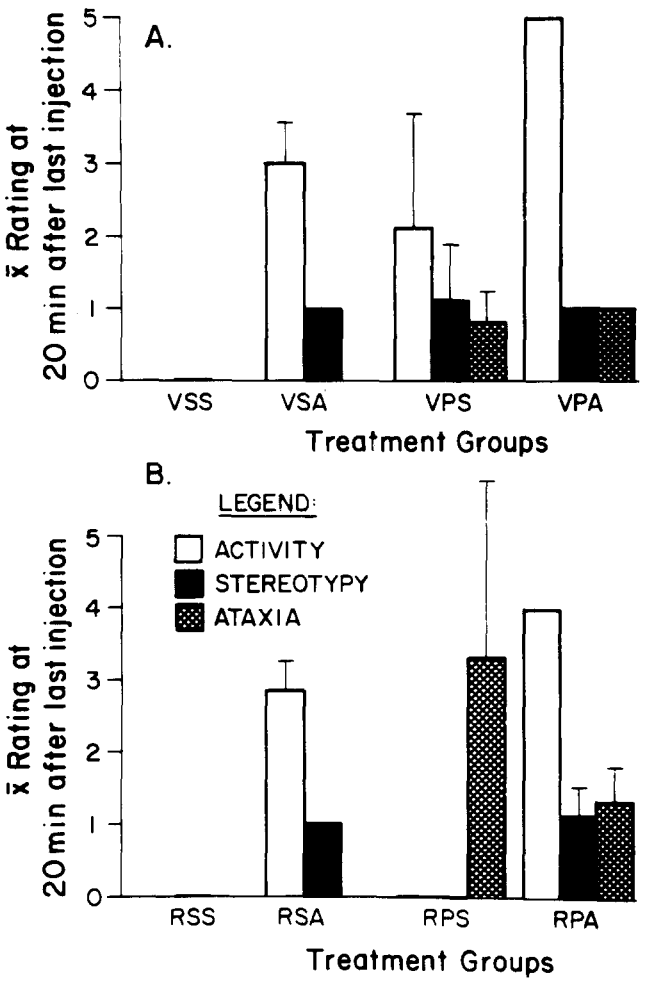

FIG. 2. Behavioral activity, stereotypy, and ataxia of subjects receiving saline (S), *d*-amphetamine (A), PCP (P), or combinations of these treatments following reserpine (R) or vehicle (V) pretreatment using the PCP-behavioral rating scale [28]. Bars indicate standard deviation. See text for time parameters.

gathered using different behavioral rating scales. The data in Fig. 1 were collected using the *d*-amphetamine rating scale reproduced in Table 1 [5]. For the data of Fig. 2, behavioral rating scales developed in this laboratory specifically for the behaviors induced by PCP were used [28]. This scale is shown in Table 2. Each subject was rated simultaneously on each rating scale by two observers in a single blind fashion (i.e., raters were unaware of the treatment conditions). For ease of presentation, reserpine, *d*-amphetamine, PCP, methylphenidate, reserpine vehicle and saline are abbreviated as R, A, P, M, V, and S, respectively, in describing treatment regimens. The combination of drugs comprising the 12 groups of this study are: VSS, VSA, VMS, VMA, RSS, RSA, RMS, RMA, VPS, VPA, RPS and RPA. Groups VSS, VSA, RSS and RSA appear in both Figs. 1 and 2. The data for these four groups in each figure were collected from the same subjects, using the simultaneous ratings on each scale as described above. Data for groups VMS, VMA, RMS and RMA appear only in Fig. 1; groups VPS, VPA, RPS and RPA appear only in Fig. 2. Thus, there were eight independent groups for which only data from the appropriate rating scale were analyzed.

TABLE 1

SCORING SYSTEM USED FOR ESTIMATION OF THE INTENSITY OF STEREOTYPY*

Score	Description of Stereotyped Behavior
0	The appearance of the animals is the same as saline treated rats
1	Discontinuous sniffing, constant exploratory activity
2	Continuous sniffing and small head movements, periodic exploratory activity
3	Continuous sniffing and small head movements, discontinuous gnawing, biting and licking. Very brief periods of locomotor activity
4	Continuous gnawing, biting and licking, no exploratory activity, occasional backward locomotion

*Reference [5].

Interrater reliability was determined for all of the behavioral ratings and ranged from 0.85 to 0.99 for all items. Statistical analysis was performed on the means of the two raters' scores. The data reported in each figure are means of 1 min ratings made 20 min after the final drug injection, and were first analyzed by a 2×4 ANOVA, after which *t*-tests were used to further analyze relevant comparisons.

All rats were injected first with either reserpine (2.5 mg/kg) or reserpine vehicle (1.0 ml/kg) at 4 hr and again at 2 hr prior to the second drug treatment. The second drug administered was methylphenidate (20.0 mg/kg), PCP (5.0 mg/kg), or saline (1.0 ml/kg). Thirty minutes following the second drug administration, either *d*-amphetamine (5.0 mg/kg) or saline (1.0 ml/kg) was injected. All drug concentrations were calculated as the salt. All injections were given intraperitoneally.

Reserpine (Serpasil®), Serpasil vehicle, and methylphenidate (Ritalin HCl®) were gifts of Ciba Pharmaceuticals Co., Summit, NJ. *d*-Amphetamine sulphate was purchased from K and K Laboratories, Inc., Plainview, NY. PCP was purchased from Bioceutics Laboratories, Inc., St. Joseph, MO.

RESULTS

The intent of this experiment was to examine the interaction of amphetamine and non-amphetamine psychomotor stimulants with the dopaminergic system through a behavioral-pharmacologic model. By recording alterations in *d*-amphetamine-induced behavior caused by PCP and methylphenidate in control or reserpine-pretreated rats, hypotheses can be suggested concerning the possible neurochemical events mediating these behaviors. To insure that it would be possible for PCP or methylphenidate to influence *d*-amphetamine-induced behaviors in a manner suggesting either an additive effect or an antagonistic effect, it was necessary to select doses of these drugs which produced behaviors rated near the midpoint of their behavioral spectrum.

Table 3 shows dose-response data for each of the three

TABLE 2

PCP BEHAVIORAL RATING SCALE*

Locomotor Activity:

0 Stationary, with little or no movement
1 Movement within localized area of cage, intermittent activities emitted at a low rate
2 Movement over a small area of cage, intermittent activity emitted at a low-moderate rate
3 Movement over small area of cage, activity emitted continuously and at a moderate-rapid rate
4 Movement over large area of cage, activity is intermittent and emitted at a low-moderate rate
5 Movement over large area of cage, activity is emitted continuously and at a moderate-rapid rate

Stereotyped Behaviors:

0 Inactive or in-place activity of a non-repetitive nature
1 Locomotor activity, sniffing and grooming more frequent than observed for control
2 Gagging, weaving, nondirected movements, occasional reciprocal forepaw treading (RFT), higher frequency of rearing or sniffing than in 1
3 Moderate rate and intermittent turning, backpeddling, praying, RFT, nondirected movements, sniffing, weaving, gagging
4 Rapid rate and continuous turning, backpeddling, praying, sniffing, weaving, gagging
5 Dyskinetic extension and flexion of limbs, head and neck, gagging and weaving

Ataxia:

0 Inactive or in-place activity, coordinated movement
1 Unusual, awkward or jerky movements, loss of balance during rearing, occasional falling on side
2 Awkward-jerky movements, moderate rate of falling on side while rearing or moving about
3 Frequent falling on back or side while moving, partial impairment of antigravity reflexes
4 Cannot move beyond a restricted area, antigravity reflexes greatly impaired, may support weight on haunches or abdomen
5 Unable to move except for twitching convulsive movements, occasional rolling on side or raising head

*Reference [28].

psychomotor stimulants used in this experiment. *d*-Amphetamine and methylphenidate were rated on the *d*-amphetamine rating scale (Table 1); PCP effects were rated on the PCP scale (Table 2). As seen, each drug induced dose-related increases in behavior. As previously reported [28], high doses of PCP produced strong behavioral ataxia which interfered with locomotor activity. From this data, *d*-amphetamine, 5.0 mg/kg, methylphenidate, 20.0 mg/kg, and PCP, 5.0 mg/kg, were selected for further investigation. These doses are approximately at the mid-point of behavioral effects induced by each drug, thus either inhibition or potentiation of the behavioral effects of these agents could be rated with the scales we used.

Overall, the 2×4 ANOVA of data collected with the *d*-amphetamine rating scale indicated that there was a significant difference in stereotyped behavior ratings between those treatment groups which received reserpine as a pretreatment and those that received the vehicle pretreatment ($p<0.001$). Results for these groups are summarized in Fig. 1a and 1b. Significant differences were also observed between the four different treatment levels (second and third drug treatment conditions) ($p<0.001$). This finding suggests that reserpine pretreatment differentially alters the effect of *d*-amphetamine and methylphenidate, and their interactions on stereotyped behavior. Examination of this effect with *t*-tests revealed that *d*-amphetamine and methylphenidate both induced significant increases in stereotyped behavior (VSS vs VSA or VMS, respectively; $p<0.01$ in both cases). Reserpine potentiated the stereotyped behavior induced by *d*-amphetamine (RSA vs VSA: $p<0.05$) and antagonized the stereotyped behavior induced by methylphenidate (RMS vs VMS; $p<0.001$). Whereas the stereotyped behavior observed in response to combined treatment with methylphenidate and *d*-amphetamine in vehicle-pretreated rats was greater than that observed in response to either drug alone (VMA vs VMS or VSA; $p<0.05$ and 0.02, respectively), in reserpine-pretreated rats methylphenidate appeared to antagonize *d*-amphetamine-induced stereotyped behavior (RMA vs RSA NS). The direction of this result is consistent with results previously reported [22,23] but the results did not reach statistical significance.

The data obtained for pretreatment groups which were rated on the PCP behavior scales are presented in Figs. 2a and 2b. Because this rating scale examines three indices of

TABLE 3

DOSE RESPONSE OF *d*-AMPHETAMINE, METHYLPHENIDATE AND PCP

d-Amphetamine		Methylphenidate		PCP			
mg/kg	Score	mg/kg	Score	mg/kg	Activity	Stereotypy	Ataxia
1.0	1.50	10	1.25	2.5	1.34	1.5	1.38
2.5	1.75	20	2.00	5.0	3.13	2.13	1.34
5.0	2.00	30	3.75	7.5	2.88	3.0	3.13
7.5	3.00	40	2.52	10.0	1.88	2.63	3.25
10.0	3.25	60	3.22	12.5	1.75	4.25	4.25
				15.0	1.50	3.83	4.38

PCP-induced behavior, the results obtained for locomotor activity, stereotyped behavior and ataxia are presented separately.

Comparison of Figs. 1 and 2 reveals that reserpine-induced potentiation of behaviors resulting from *d*-amphetamine administration were reflected only by the *d*-amphetamine rating scale. Because the PCP scales were designed to quantify the unique behaviors induced by PCP, the inability of these scales to monitor the relatively small changes in *d*-amphetamine-induced behavior observed here is probably a function of their intended bias toward PCP-induced behaviors.

Locomotor Activity

Overall 2×4 ANOVA indicated that groups receiving reserpine pretreatment displayed significantly less locomotor activity than saline-pretreated subjects ($p<0.001$). A significant difference was also observed between the different treatment levels ($p<0.01$) and in the interaction between these treatments and reserpine pretreatment ($p<0.001$). *t*-Test analysis of these significant effects indicated that *d*-amphetamine and PCP both induced significant increases in locomotor activity using this rating scale (VSS vs VSA and VPS, $p<0.001$ in both cases). Contrary to the results obtained with the *d*-amphetamine rating scale, reserpine treatment did not potentiate *d*-amphetamine-induced activity (RSA vs VSA: NS). Reserpine did, however, completely antagonize PCP-induced locomotor activity (RPS vs VPS: $p<0.001$). The combined effects of *d*-amphetamine and PCP on locomotor activity were significantly greater than the individual effects of either drug in vehicle pretreated rats (VPA vs VSA and VPS; $p<0.01$ in both cases) and in reserpine pretreated rats (RPA vs RSA and RPS; $p<0.05$ and $p<0.001$, respectively).

Stereotyped Behavior

For the stereotyped behavior measure, overall significant differences were observed for reserpine pretreatment ($p<0.01$), drug treatment combination ($p<0.001$) and the drug combination interaction with reserpine pretreatment ($p<0.01$). Subsequent *t*-tests indicated that *d*-amphetamine and PCP both induced significant increases in stereotyped responses (VSS vs VSA and VPS; $p<0.001$ and $p<0.05$, respectively). Similar to the interaction seen in locomotor activity, reserpine pretreatment abolished PCP-induced stereotyped behavior (RPS vs VPS; $p<0.001$) but the stereotyped responses observed in subjects receiving both *d*-amphetamine and PCP with reserpine pretreatment were approximately equal to those seen in subjects receiving only *d*-amphetamine with reserpine pretreatment (RPA vs RSA; NS) or subjects receiving *d*-amphetamine and PCP after vehicle pretreatment (RPA vs VPA; NS).

Ataxia

Overall ANOVA indicated that reserpine-pretreated subjects were significantly more ataxic than vehicle-pretreated subjects ($p<0.001$). A significant difference was also observed between the treatment combinations of PCP and *d*-amphetamine ($p<0.001$) and between their interaction with reserpine-pretreatment ($p<0.001$). *t*-Test analysis, subsequent to the ANOVA, indicated that reserpine pretreatment greatly potentiated PCP-induced ataxia (RPS vs VPS; $p<0.001$), which could contribute to the inhibition by reserpine of PCP-induced locomotor activity and stereotyped behavior. *d*-Amphetamine did not significantly alter PCP-induced ataxia in vehicle-pretreated subjects (VPS vs VPA; NS), but subjects receiving *d*-amphetamine and PCP following reserpine-pretreatment had reduced ataxia in comparison to reserpine-PCP-saline subjects (RPA vs RPS; $p<0.05$).

DISCUSSION

The stereotyped behaviors induced by PCP are qualitatively different from those induced by methylphenidate and *d*-amphetamine. The effects of the latter two drugs are quite similar and can both be rated on a scale developed for *d*-amphetamine [5]. In order to accurately represent the effects of all three drugs, we utilized a scale specifically designed for PCP-induced behaviors [28] as well as that developed for *d*-amphetamine-induced behaviors [5] to quantitate the behavioral effects of various-combinations of PCP, methylphenidate and *d*-amphetamine. Use of two different scales introduced some difficulty in data interpretation. however, in that the PCP rating system records locomotor activity, stereotyped behavior and ataxia on separate scales, whereas the *d*-amphetamine scale progresses from locomotor activity (low rating scores) through stereotyped behavior (high rating scores) on one scale. Behavioral ataxia is not rated on the *d*-amphetamine scale, thus compromising its usefulness in rating the behaviors which result when PCP is administered together with *d*-amphetamine or methylphenidate. This further indicates the necessity of quantifying PCP-induced behaviors with rating scales which allow the separate rating of ataxia, such as the PCP rating scale we have developed [28].

It is possible that some of the results reported here are

due to alterations in drug absorption or metabolism, or through interactions between drugs in animals administered multiple drugs. This must particularly be considered in reserpine-pretreated subjects where methylphenidate antagonized *d*-amphetamine-induced behaviors, and in reserpine or vehicle pretreated rats where PCP potentiated the effects of *d*-amphetamine. Methylphenidate antagonism of *d*-amphetamine effects in reserpine pretreated rats is supported by similar antagonism in *in vitro* experiments [21]. Further study will be needed to rule out the possibility that alterations in levels of amphetamine or its metabolites in brain account for the observed interaction effects with PCP.

Overall, the results reported above suggest greater similarity between the mechanism of action of PCP and methylphenidate than between PCP and *d*-amphetamine, even though the behavioral effects of methylphenidate and *d*-amphetamine are more similar to each other than to PCP. The effects of methylphenidate on stereotyped behavior and the effects of PCP on locomotor activity and stereotyped behavior are both inhibited by pretreatment with a dose of reserpine which potentiates the effect of *d*-amphetamine on stereotyped behavior. Also, just as methylphenidate potentiates *d*-amphetamine-induced stereotyped behavior in vehicle-pretreated subjects, PCP potentiates the effects of *d*-amphetamine on locomotor activity in vehicle-pretreated subjects. Similar results have recently been reported by Balster and Chait [1]. However, methylphenidate slightly antagonized the effect of *d*-amphetamine on stereotyped behaviors in reserpine-pretreated subjects, whereas PCP potentiated the *d*-amphetamine effect on locomotor activity in this group. This indicates that PCP must have some neurochemical effects which differ from those of methylphenidate.

PCP administered in combination with *d*-amphetamine did not result in greater stereotypy scores than PCP alone. Although the reason for this apparent discrepancy with the locomotor activity ratings is not immediately apparent, it is possible that this result is an artifact of the two scales. Since methylphenidate and *d*-amphetamine combinations were rated on a scale which was accurate for the behaviors induced by both drugs, an increase resulting from combined administration would be easily detected. However, PCP and *d*-amphetamine combinations were rated on a scale designed for behaviors induced by PCP. Because most of the stereotypies induced by *d*-amphetamine are rated on the lower ratings on the PCP-stereotypy scale, increased stereotypies of the amphetamine type would not be recorded.

Analysis of the mechanisms of action for *d*-amphetamine, methylphenidate, and PCP suggests the following hypotheses to explain these results. *d*-Amphetamine has been reported to gain entry into DA neurons either via diffusion or through a carrier-mediated exchange diffusion [10,19]. As *d*-amphetamine enters the neuron through this carrier-mediated exchange process, it simultaneously increases the transfer of newly synthesized DA to the extra-neuronal space, down the DA concentration gradient. Once it is extra-neuronal, uptake of DA is competitively inhibited by the high extra-neuronal concentration of *d*-amphetamine. Reserpine potentiates the effect of *d*-amphetamine presumably by blocking storage of vesicular DA, thereby increasing the intraneuronal DA pool available for release. Thus, in vehicle-pretreated rats, *d*-amphetamine may act via enhanced release of newly synthesized DA and blockade of DA reuptake, but in reserpine-pretreated rats it may also facilitate the release of cytoplasmic DA that was originally in the storage pool.

As previously stated, methylphenidate effectively antagonizes uptake of catecholamines [8,21], an effect which may contribute to its stimulant properties. Others have proposed that methylphenidate induces its behavioral effects through the release of DA from a carrier-independent, vesicular storage pool [4, 6, 25]. According to this theory, reserpine antagonizes the stimulant effect of methylphenidate by depleting the storage vesicles of DA, thereby reducing the amount of DA available for release. The potentiation of stereotyped behavior we observed in response to combined treatment with *d*-amphetamine and methylphenidate is consistent with the additive effects of two independent release mechanisms; the blockade of this additive effect by reserpine is again consistent with the removal of the methylphenidate-induced release of vesicular DA. Under these conditions, *d*-amphetamine could still release newly synthesized DA, and both drugs could block its uptake. Alternatively, it has been proposed that methylphenidate could antagonize the action of *d*-amphetamine in reserpinized rats by blocking its uptake into the neuron or by blocking the subsequent release of DA [22]. The former possibility is inconsistent with the demonstration that methylphenidate did not antagonize *d*-amphetamine-induced depletion of dihydroxyphenylacetic acid (DOPAC) levels in reserpinized rats [11] at a dose sufficient to induce behavioral effects. Moreover, *d*-amphetamine can enter the neuron to equilibrium concentrations solely on the basis of diffusion [10]. Thus, these reports suggest that methylphenidate inhibits the *d*-amphetamine-induced release of DA into the synaptic cleft.

Assuming the validity of these models, our results suggest that PCP acts primarily through release of reserpine-sensitive, vesicular DA, hence the blockade of its action following reserpine pretreatment. Like methylphenidate and *d*-amphetamine, PCP can also inhibit DA reuptake [26,27], but our data suggest that, unlike methylphenidate, it cannot block the entry of *d*-amphetamine-released DA into the synaptic cleft. This was also apparent from the difference in the effects of methylphenidate and PCP on *d*-amphetamine-induced behaviors in reserpine-pretreated rats. It is unlikely that the PCP-induced potentiation of amphetamine-induced locomotor activity in vehicle- or reserpine-pretreated rats is the result of direct DA-agonist properties [8]. Furthermore, PCP does not effectively antagonize ^{14}C-DA binding to rat striatal membranes [14] nor does it antagonize ^{3}H-spiroperidol binding to rat striatal membranes (Meltzer and So, unpublished data). Like *d*-amphetamine, but unlike methylphenidate, PCP may also enhance the release of newly synthesized DA since some of its effects are blocked by AMPT [8,9]. In limited biochemical testing of the ability of PCP to enhance release of ^{3}H-DA from striatal slices, PCP was only weakly active (Doherty *et al.*, in preparation) but the method employed does not preclude the possibility that impulse-induced release of DA might be enhanced by PCP.

It must be kept in mind that reserpine potentiates PCP-induced ataxia (Fig. 2). Although it does not negate the above discussion of proposed neurochemical events mediating these effects, it is possible that this increased ataxia could have antagonized PCP-induced locomotor activity in a manner independent of these mechanisms.

Table 4 presents a model of the relative importance of presynaptic mechanisms mediating the effects of *d*-amphetamine, methylphenidate, and PCP. We suggest that the primary effect of PCP is to release DA from a reserpine-sensitive, vesicular pool. In this respect, PCP would appear

TABLE 4

COMPARISON OF PRE-SYNAPTIC EFFECTS OF *d*-AMPHETAMINE, METHYLPHENIDATE AND PCP ON DOPAMINE NEURONS

	Release of Reserpine-Sensitive DA	Release of AMPT-Sensitive DA	Blockade of *d*-Amphetamine-Induced Release Into Synaptic Cleft	DA Uptake Blockade
d-Amphetamine	+	+++	Not Applicable	++
Methylphenidate	+++	+	++	++
PCP	+++	+	0	++

to be more similar to methylphenidate than to *d*-amphetamine. PCP also appears to weakly antagonize DA uptake, an effect common to both *d*-amphetamine and methylphenidate. PCP may also release newly synthesized DA but is much less potent in this respect than *d*-amphetamine. Whereas methylphenidate appears to antagonize *d*-amphetamine-induced release of DA into the synaptic cleft, PCP does not appear to share this effect. It seems probable that the ability of PCP to release newly synthesized DA, compared to its ability to release DA from a vesicular pool, is relatively weak, and is therefore clearly demonstrable only under conditions designed to minimize release from the vesicular pool (i.e., reserpine-pretreatment). Further *in vitro* and *in vivo* studies designed to analyze conditions under which PCP can increase impulse-dependent DA release from vesicular stores appears indicated.

This study has focused on an analysis of what appear to be dopaminergic effects of PCP. We have discussed elsewhere that the pharmacology of PCP is quite complex and that effects on noradrenergic, cholinergic, and serotonergic systems must also be considered to fully explain the complicated set of effects of PCP. The effects of PCP on these other neurotransmitters are reviewed elsewhere [8, 9, 15, 26, 27, 28].

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