Behavioral and Neurochemical Interactions of Dextroamphetamine and Methylphenidate in Rats

T. G. REIGLE *x, W. L. ISAAC ‡, and W. ISAAC ‡

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Abstract □ The effects of dextroamphetamine and methylphenidate on locomotor activity and brain levels of norepinephrine and dopamine were compared in male Sprague–Dawley rats. Both drugs produced a dose-related increase in locomotor activity during the hour immediately following intraperitoneal administration. However, combined administration of the drugs elicited only the effect of dextroamphetamine. Brain levels of norepinephrine and dopamine also increased 1 hr after dextroamphetamine dosing. Methylphenidate did not exhibit these effects and antagonized the neurochemical changes produced by dextroamphetamine. Although both drugs are considered to exert their effects by indirect activation of brain catecholamine systems, differences in their mechanism of action appear to result in a lack of additive or antagonistic effects when dextroamphetamine and methylphenidate are coadministered. These findings may have clinical significance with respect to the use of such agents in minimal brain dysfunction.

Keyphrases □ Stimulants—dextroamphetamine and methylphenidate, effects on behavior and neurochemistry, rats □ Dextroamphetamine—effects on behavior and neurochemistry, rats, coadministration with methylphenidate □ Methylphenidate—effects on behavior and neurochemistry, rats, coadministration with dextroamphetamine □ CNS agents—dextroamphetamine and methylphenidate, effects on behavior and neurochemistry, rats

Both dextroamphetamine and methylphenidate have been employed successfully in the treatment of hyperkinesis or minimal brain dysfunction (1, 2). In experimental animals, these drugs exhibit the characteristic actions of central nervous system (CNS) stimulants by producing dose-dependent increases in locomotor activity and stereotyped behaviors (3, 4). Both the clinical and behavioral stimulant actions of these agents have been attributed to activation of brain catecholamine systems (5, 6). However, the mechanisms by which these drugs enhance brain catecholamine activity apparently are different.

BACKGROUND

Reserpine, which depletes catecholamines by interfering with presynaptic granular storage, antagonizes the stimulant effects of methylphenidate but not those of dextroamphetamine (7). α-Methyl-*p*-tyrosine, an inhibitor of catecholamine synthesis, antagonizes the effects of dextroamphetamine but leaves the actions of methylphenidate relatively intact (8). These findings led to the proposal that methylphenidate facilitates the impulse-mediated release of catecholamines from reserpine-sensitive granular storage pools whereas dextroamphetamine acts primarily by increasing the release of a reserpine-resistant pool of newly synthesized cytoplasmic amines. Other investigators (9, 10) suggested that methylphenidate acts by blocking the presynaptic uptake of catecholamines released by nerve activity and that dextroamphetamine acts primarily by enhancing catecholamine release in the absence of neuronal stimulation.

Since both drugs are individually effective in ameliorating hyperkinetic symptoms, produce similar behavioral effects in animals, and indirectly activate brain catecholamine systems by different mechanisms, it was felt that coadministration of these agents might potentiate their separate effects and have possible clinical significance. This hypothesis was tested by evaluating the effects of moderate doses of dextroamphetamine and methylphenidate, individually and in combination, on the locomotor activity and brain catecholamine content of rats. The dose levels of both drugs were shown to have submaximal effects (3, 11, 12) and, thus, permitted the appearance of behavioral or neurochemical changes in either direction upon combination.

EXPERIMENTAL

Animals—Sprague–Dawley male albino rats[1] were 20 days old when received and were adapted to the laboratory and daily handling for 7 days. For an additional 15 days, they were adapted to the apparatus and handling and injection procedures (13). Food and water were available at all times, and a 12-hr light–dark cycle was maintained.

Apparatus—The rats were housed in clear plastic mouse-breeding cages (45 × 24 × 25 cm) with a hardware cloth cover during activity measurements. Each plastic container was housed in a separate sound-treated cubicle, open at one end; it was 85 × 56 × 56 cm and individually lighted with a 20-w fluorescent bulb mounted 29 cm above the floor of the activity chamber. Twelve such cubicles were housed in a sound-treated room.

A single IR light beam bisected the length of each plastic container ~2 cm above the floor and fell upon a photocell. An IR filter covered the photocell, making it insensitive to ambient illumination. When the subjects broke the beam in their chambers, counts were recorded on individual counters in a separate room.

Procedure—Two dose levels of methylphenidate hydrochloride (1.6 and 3.2 mg/kg) and dextroamphetamine sulfate (0.4 and 0.8 mg/kg) and a combination of dextroamphetamine sulfate (0.4 mg/kg) and of methylphenidate hydrochloride (1.6 mg/kg) were studied. Placebo injections of the drug vehicle (bacteriostatic water) were included to give a total of six experimental conditions. All injections were administered intraperitoneally in an equal volume (1 ml/kg) 5–10 min before the subjects were placed in the activity measurement apparatus. When placed in the cubicle, all equipment was turned on and 5 min was allowed for adaptation. Activity measures were obtained for the next 60 min. Activity scores were transformed by a $\sqrt{x+1}$ transformation, as recommended for frequency scores (14).

Six separate orders of daily drug administrations were utilized, producing a 6 × 6 Latin square with two subjects within each order. All subjects were run through their orders three times. The first replication was for adaptation to the drug injections, and only scores from the last two replications were compared and evaluated by analysis of variance and Duncan's multiple range test (14).

Age-matched rats subjected to adaptation and handling procedures identical to those already described (13) were used for brain catecholamine analysis. Groups of animals (eight to 10 rats/group) received the same drug treatments utilized in the locomotor activity study and were sacrificed 30 min after intraperitoneal injection. Whole brains were removed rapidly, rinsed with tap water, and homogenized[2] in 12 ml of cold 0.4 *N* $HClO_4$. Homogenates were centrifuged at 30,000×*g* for 30 min, and the supernate was frozen immediately and stored for 1–2 days prior to chemical assay. Norepinephrine and dopamine were assayed by the fluorometric methods of Anton and Sayre (15) and Carlsson and Waldeck (16), respectively. Catecholamine concentrations were expressed as nanograms per gram of brain (wet weight) and were compared across treatments by analysis of variance and Duncan's multiple range test (14).

RESULTS AND DISCUSSION

The locomotor activity data are presented in Fig. 1. Analysis of variance revealed that the difference between replications was not significant [$F(1,11) = 3.94$, $p > 0.05$], nor was the interaction between drug conditions and replications [$F(5,55) = 1.25$, $p > 0.05$]. The activity levels observed under the drug conditions, however, did differ significantly

[1] Southern Animal Farms, Prattville, Ala.
[2] Brinkmann Polytron homogenizer PT10-35.

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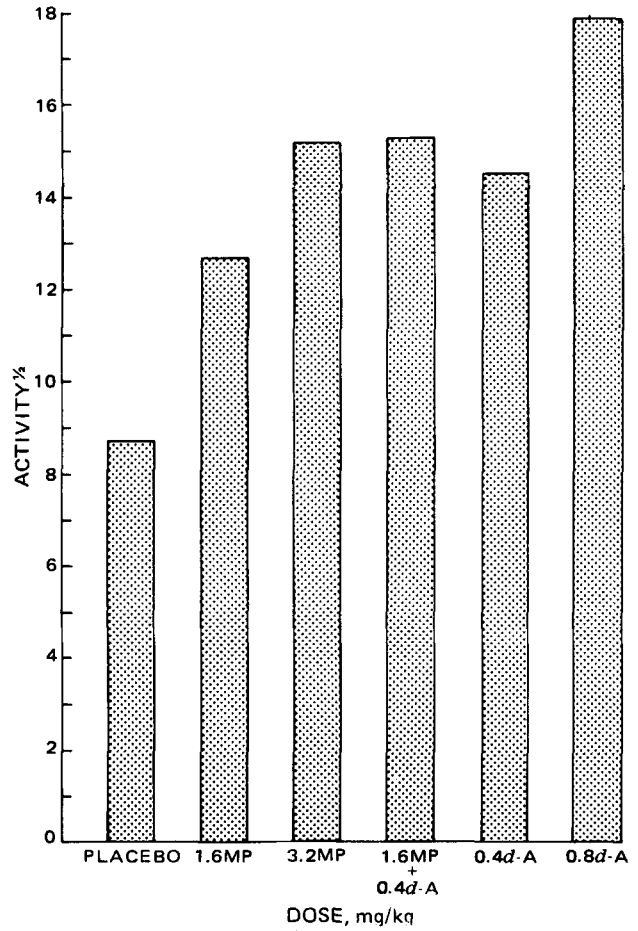

Figure 1—*Effects of dextroamphetamine (d-A), methylphenidate (MP), and their combination on square root-transformed locomotor activity scores in the rat.*

[$F(5,55) = 55.82$, $p < 0.01$]. Further analysis of the differences among drug treatments using the multiple range test ($\alpha = 0.01$) indicated that the placebo condition produced the lowest amount of locomotor activity and that the low dose of methylphenidate produced a significant increase. Administration of 0.4 mg of dextroamphetamine/kg, 3.2 mg of methylphenidate/kg, and the combined dose of 0.4 mg of dextroamphetamine/kg and 1.6 mg of methylphenidate/kg produced increases in locomotor activity that were significantly greater than the lower dose of methylphenidate, but the effects of these treatments did not differ significantly among themselves. The highest dose of dextroamphetamine produced a higher level of activity than the other drug conditions.

Thus, both drugs produced dose-related increases in locomotor activity and, as demonstrated previously (3), dextroamphetamine was more potent than methylphenidate. However, the drug combination was no more effective than dextroamphetamine alone. Intermediate dose levels of both drugs (3) were chosen so that changes in activity in either direction, as a consequence of the drug combination, could be detected. Since the higher dose of dextroamphetamine produced a greater increase in activity than the other treatments, the effects of the dextroamphetamine–methylphenidate combination were not limited by an activity ceiling. Also, since both the placebo and low dose of methylphenidate produced less activity than the drug combination, the results were not limited in the opposite direction. With these doses, methylphenidate neither enhanced nor suppressed the effects of dextroamphetamine on locomotor activity.

The effects of the various drug treatments on brain catecholamine levels are presented in Table I. Analysis of variance revealed significant differences among treatments on the brain levels of both norepinephrine [$F(5,51) = 5.14$, $p < 0.01$] and dopamine [$F(5,46) = 12.53$, $p < 0.01$]. Further analysis of these differences (Duncan's multiple range test; $\alpha = 0.05$) indicated that both doses of dextroamphetamine produced significant increases in brain dopamine, although the effects of these treatments were not different from each other. Brain dopamine levels following either methylphenidate or the dextroamphetamine–methylphenidate combination were not significantly different from those observed in the placebo-treated animals. Similar findings were obtained with respect to brain norepinephrine. The increase produced by 0.8 mg of dextroamphetamine/kg did not quite achieve statistical significance ($0.05 < p < 0.10$). However, the 0.4-mg/kg dose significantly increased brain norepinephrine, and the effects of the two amphetamine doses did not differ from each other. Once again, the effects of all other treatments were not significantly different from those of the placebo.

Table I—Effects of Dextroamphetamine, Methylphenidate, and Their Combination on Rat Brain Catecholamines

Treatment (Dose, mg/kg)	Dopamine		Norepinephrine	
	Nanograms per Gram[a]	Percent Placebo	Nanograms per Gram[a]	Percent Placebo
Placebo	760 ± 14	—	287 ± 10	—
Dextroamphetamine (0.4)	894 ± 23[b]	118[b]	325 ± 12[c]	113[c]
Dextroamphetamine (0.8)	924 ± 29[b]	122[b]	320 ± 12[d]	111[d]
Methylphenidate (1.6)	771 ± 13	101	287 ± 10	100
Methylphenidate (3.2)	788 ± 24	104	257 ± 13	90
Dextroamphetamine (0.4) + methylphenidate (1.6)	769 ± 19	101	272 ± 13	95

[a] Data expressed as mean ± *SEM* of eight to 10 animals/group. [b] $p < 0.01$ *versus* placebo. [c] $p < 0.05$ *versus* placebo. [d] $p < 0.10$ *versus* placebo.

Thus, with respect to these neurochemical measures, not only were the effects of dextroamphetamine not enhanced by methylphenidate, they were significantly antagonized. The effects of amphetamine on brain catecholamine systems are complex. However, in agreement with the present findings, most previous reports indicated that low amphetamine doses capable of increasing locomotor activity produced increases in brain norepinephrine and dopamine while higher doses tended to cause a depletion (11, 12). In contrast, doses of methylphenidate that produced comparable increases in locomotor activity had no effect on brain catecholamines, indicating a difference in the mechanism of action of these two drugs. The doses of amphetamine employed could increase brain catecholamine levels by stimulating synthesis, inhibiting metabolism, or decreasing neuronal firing. The ability of amphetamine to inhibit monoamine oxidase has been demonstrated *in vitro*; however, rather large concentrations were required, and enzyme inhibition was difficult to observe following *in vivo* drug administration (17). Impulse flow in catecholamine neurons is inhibited by amphetamine (18), but this action also is produced by several other drugs, including methylphenidate, that have no appreciable effect on brain catecholamine levels (8).

Thus, the most plausible explanation for increases in brain catecholamines following amphetamine appears to be synthesis enhancement. The increased release elicited by amphetamine of newly synthesized catecholamines from nongranular cytoplasmic pools could reduce end-product inhibition of cytoplasmic tyrosine hydroxylase in presynaptic neurons (19). This would result in the increased formation and, hence, elevated levels of brain catecholamines observed in the present study. The greater release produced by larger amphetamine doses could exceed the synthetic capacity of the affected neurons, resulting in the net catecholamine depletion observed previously (11, 12).

The observed antagonism of amphetamine-induced increases in brain catecholamines by methylphenidate could be explained similarly. By facilitating the efflux of catecholamines from granular storage, methylphenidate would provide a source of replacement for the amines released from cytoplasmic pools by amphetamine such that synthesis could not be activated by a reduced catecholamine level in the cytoplasmic compartment. Behaviorally, this would not prevent stimulant effects due to the release of newly synthesized catecholamines by amphetamine but could prevent an additive action by methylphenidate since the amines displaced from the granules might replenish the cytoplasmic pool rather than act as a source for the activation of postsynaptic events.

Although amphetamine and methylphenidate individually exert behavioral stimulant effects and enhance the activity of brain catecholamine systems, differences in their mechanism of action can prevent additive or potentiating effects when these drugs are coadministered. These findings suggest that a therapeutic advantage may not be gained by combining amphetamine and methylphenidate in the treatment of hyperkinetic disorders.

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Plasma Protein Binding of Zomepirac Sodium

PATRICK J. O'NEILL

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Abstract □ The plasma protein binding of zomepirac, a new nonnarcotic analgesic, was studied using equilibrium dialysis. Experiments were performed using human plasma and plasma from mice, rats, and rhesus monkeys, all species of pharmacological or toxicological interest. At concentrations approximating those achieved *in vivo*, the binding was fairly constant at 98–99% in all species except the rhesus monkey, where binding was decreased from 98 to ~96% at higher concentrations (>50 μg/ml). Zomepirac (10 μg/ml) did not appear to displace or to be displaced by warfarin (10 μg/ml) in human plasma. However, salicylate (5–200 μg/ml) caused a concentration-dependent decrease in zomepirac (10 μg/ml) binding. Zomepirac did not affect salicylate binding.

Keyphrases □ Zomepirac sodium—plasma protein binding studies in plasma from humans, rats, mice, and rhesus monkeys, interaction studies with warfarin and salicylic acid □ Plasma protein binding—zomepirac sodium in plasma from humans, rats, mice, and rhesus monkeys □ Warfarin—interaction studies with zomepirac sodium, effect on plasma protein binding □ Salicylic acid—interaction studies with zomepirac sodium, effect on plasma protein binding □ Analgesics, nonnarcotic—zomepirac sodium, plasma protein binding studies in humans, rats, mice, and rhesus monkeys, interaction studies with warfarin and salicylic acid

Plasma protein binding of drugs is an important factor in drug disposition (1–3). This report describes the interaction of zomepirac sodium, a new nonnarcotic analgesic agent (4–6), with plasma proteins from several species. Some preliminary binding interaction studies in human plasma are also reported.

EXPERIMENTAL

Materials—Zomepirac sodium [sodium 5-(4-chlorobenzoyl)-1,4-dimethyl-1*H*-pyrrole-2-acetate dihydrate] was labeled with carbon 14 at a specific activity of 10.18 μCi/mg[1]. The drug was 96–98% radiochemically pure by TLC at the time of use. [^{14}C]Salicylic acid[2] and [^{14}C]warfarin[2] were used at specific activities of 421 and 164 μCi/mg, respectively. Both drugs were 99% radiochemically pure by TLC.

Heparinized plasma was harvested from blood collected from Wistar rats[3], CD-1 Swiss mice[3], and rhesus monkeys[4]. Citrated human plasma was purchased locally. Sorensen's buffer (0.067 *M* phosphate, pH 7.4) was prepared by dissolving 1.72 g of monobasic potassium phosphate and 7.70 g of dibasic sodium phosphate in 1 liter of distilled water.

Methods—Binding experiments were performed on an equilibrium dialysis system[5]. Regenerated cellulose dialysis membranes (mol. wt. cutoff of 5000) were prepared by three rinses in distilled water and three rinses in buffer. The membranes were placed between two-piece polytef dialysis cells (1-ml volume per side) mounted in a spring-loaded rack, and each half-cell was filled. One side of the cell was filled with plasma, and the other side was filled with the drug in buffer.

The cells were rotated at 12 rpm for 2 hr at room temperature, and then the half-cells were emptied and assayed for total carbon 14 as a measure of zomepirac. These dialysis conditions were selected since preliminary experiments in the absence of plasma (*i.e.*, zomepirac dialyzed against buffer) showed that equilibrium was reached in 2 hr and that plasma binding was not different at room temperature or 37°. Furthermore, TLC analysis of selected samples showed that no degradation of zomepirac occurred under these conditions.

Interaction Studies—[^{14}C]Zomepirac was dialyzed against undiluted rat, mouse, monkey, and human plasma at concentrations ranging from 0.1 to 250 μg/ml, depending on the species. These concentrations were in the ranges of those observed after pharmacological doses of zomepirac sodium (7–10).

To assess the potential for displacement of zomepirac by other agents, experiments were performed with warfarin[6] and salicylic acid[7] using human plasma. The [^{14}C]zomepirac concentration was 10 μg/ml. Warfarin was tested at 10 μg/ml, and salicylic acid was tested from 5 to 200 μg/ml. When the effect of zomepirac on the binding of these agents was evaluated, a tracer of [^{14}C]warfarin (~10,000 dpm/ml, 0.03 μg/ml) or salicylate (~10,000 dpm/ml, 0.01 μg/ml) was added, and nonradioactive zomepirac was used.

Sample Analysis—Total radioactivity was determined by adding aliquots of both plasma and buffer solutions (after dialysis) to 10 ml of scintillation cocktail[8]. Samples were counted in a refrigerated liquid scintillation spectrometer and were corrected for quenching using the external standard method.

Selected samples were analyzed for drug decomposition during dialysis. Zomepirac was assayed by applying aliquots of the postdialysis plasma and buffer samples to silica gel TLC plates[9] developed in chloroform–methanol–acetic acid (94:5:1 v/v/v). Analysis was by either segmentation and liquid scintillation counting or radioscan. No decomposition was observed.

[1] McNeil Pharmaceutical, Spring House, Pa.
[2] Amersham Corp., Des Plaines, Ill.
[3] Charles River, Wilmington, Mass.
[4] Primate Imports, Port Washington, N.Y.
[5] Dianorm, Diachema Ag., Ruschlikon, Switzerland.
[6] Endo Laboratories, Garden City, N.Y.
[7] J. T. Baker Chemical Co., Phillipsburg, N.J.
[8] Biofluor, New England Nuclear, Boston, Mass.
[9] GF 254 (250 μm), Analtech, Newark, Del.

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