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Comparison of 2,5-Dimethoxy-4-Methylamphetamine (DOM) and d-Amphetamine for In Vivo Efflux of Catecholamines from Rat Brain[1]

J. J. VRBANAC, H. A. TILSON,[2] K. E. MOORE AND R. H. RECH

Department of Pharmacology, Michigan State University, East Lansing, Michigan 48824

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VRBANAC, J. J., H. A. TILSON, K. E. MOORE AND R. H. RECH. *Comparison of 2,5-dimethoxy-4-methylamphetamine (DOM) and d-amphetamine for in vivo efflux of catecholamines from rat brain.* PHARMAC. BIOCHEM. BEHAV. 3(1) 57–64, 1975. – The neurochemical effects of DOM and *d*-amphetamine were compared under several conditions in unanesthetized rats implanted with chronic-indwelling push-pull cannulae in a cerebral lateral ventricle. Brain catecholamine storage sites were previously pulse-labelled with ^{14}C-norepinephrine administered intraventricularly. During the perfusion of the lateral ventricles with artificial cerebrospinal fluid, the animals were injected i.p. with 1.5 mg/kg of DOM, 2.0 mg/kg of *d*-amphetamine or 1.0 ml/kg of isotonic saline. Analysis of the perfusate in successive samples indicated an increased efflux of ^{14}C-radioactivity in rats administered DOM or *d*-amphetamine. Increased proportions of ^{14}C-norepinephrine and ^{14}C-normetanephrine were detected in samples of perfusate 15 to 30 min after drug injection. Pretreatment of other animals with 6-hydroxydopamine intraventricularly, which decreased brain levels of both norepinephrine and dopamine, blocked the increased efflux of ^{14}C-radioactivity induced by DOM or *d*-amphetamine. Pretreatment of rats with 6-hydroxydopa i.p., which depleted brain norepinephrine selectively, reduced to about half the *d*-amphetamine-induced efflux of ^{14}C-radioactivity for all samples during the time course of the effect. However, animals pretreated with 6-hydroxydopa and then tested for DOM effects showed a different pattern of ^{14}C-radioactivity efflux. The efflux for the initial samples was increased as with the DOM control, but the 6-hydroxydopa pretreatment attenuated the DOM-induced efflux for the later samples. The results suggest DOM and *d*-amphetamine share qualitatively similar effects in releasing and/or blocking the reuptake of catecholamines at brain periventricular nerve terminals. Nevertheless, DOM appears to differ from *d*-amphetamine in the temporal pattern of net catecholamine release.

2,5-Dimethoxy-4-methylamphetamine d-Amphetamine Catecholamine efflux Ventricular perfusion Push-pull cannulas 6-Hydroxydopamine 6-Hydroxydopa

A NUMBER of methoxylated amphetamine derivatives have been synthesized and their pharmacological effects investigated [15]. One of these, 2,5-dimethoxy-4-methylamphetamine (DOM), has been reported to produce in humans a mild euphoria and enhanced self-awareness in lower dosages (2–3 mg) and sensory alterations after higher doses (5 mg; [16]). The subjective effects of lower doses of DOM and its ethyl homologue (2,5-dimethoxy-4-ethylamphetamine) reportedly share some common characteristics with a 10 mg dose of *d*-amphetamine [16]. Furthermore, recent studies have indicated that lower doses of DOM and *d*-amphetamine produce a similar behavioral profile in a signalled avoidance paradigm [2], as well as producing similar rate-dependent effects on other types of schedule-controlled behavior [10,21].

DOM appears to pass into the central nervous system with ease and concentrate in specific brain regions, but brain monoamine concentrations are influenced only by relatively large doses [6, 7, 9, 25]. These observations seem at odds with the proposal that an altered disposition of brain catecholamines (CA) is implicated in the mechanism of action of several classes of psychoactive agents [19]. The purpose of the present investigation was to explore more fully the effects of DOM on brain CA of rats, using an in vivo brain perfusion technique, and to compare these effects with those of *d*-amphetamine.

METHOD

Ventricular Perfusion Studies

Nine male albino rats (Sprague-Dawley strain, Spartan Farms, Haslett, Mich.), weighing approximately 300 g, were

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[2] Present address: Pharmacology Department, Bristol Laboratories, Syracuse, New York 13201.

housed in groups of 3 in air-conditioned, temperature-controlled quarters. The animals were divided randomly into 3 equal groups. The first group was anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and injected intraventricularly with 6-hydroxydopamine hydrobromide (Regis Chemical Co., Chicago, Ill.; 252 μg, calculated as the base; this group is designated 6-OHDA). The 6-OHDA was dissolved in isotonic saline (NaCl) containing ascorbic acid (1 mg/ml), a total volume of 25 μl being infused into the right lateral ventricle over a 3 min period by means of an infusion pump. The infusion was facilitated by mounting the rats in a D. H. Kopf stereotaxic instrument and lowering a 30 ga needle through a craniotomy 3.5 mm into the brain at 1 mm lateral to midline and 1 mm posterior to the bregma. The second group of animals was subjected to the same procedure except that the ascorbic acid vehicle without 6-OHDA was infused into the ventricle (this group is designated Control). The third group remained untreated at this time.

Two weeks later all rats were anesthetized with sodium pentobarbital and a push-pull cannula was implanted chronically into each right lateral ventricle, using the coordinates described for the intraventricular infusions. Physical dimensions of the cannula and the method of implantation have been reported in detail elsewhere [20].

Two weeks after implantation of the cannulae the third group of rats was injected i.p. with 40 mg/kg of the 6-hydroxy-derivative of dihydroxyphenylalanine (Regis Chemical Co.) suspended in 0.5% methyl cellulose containing 1% ascorbic acid (this group is designated 6-OHDOPA). This treatment was preceded 2 and 18 hr, respectively, by i.p. injections of 50 mg/kg of β-(3,4-dihydroxyphenyl)-α-hydrazine-α-methyl-DL-proprionic acid (HMD, a peripheral decarboxylase inhibitor) suspended in aqueous methyl cellulose and 10 mg/kg of tranylcypromine sulfate dissolved in NaCl. At least 3 weeks intervened between this treatment and the perfusion experiments described below.

One hr prior to ventricular perfusion, each animal received a slow infusion of 10 μl of ^{14}C-*d,l*-norepinephrine (New England Nuclear, 0.38 μg/μl, 1.0 μC) into the right lateral ventrical via the inner tube of the push-pull cannula. This large amount of catecholamine, relative to what would constitute a tracer dose, was chosen on the basis of previous experience with intraventricularly administered ^{14}C-5-hydroxytryptamine [20]. Such a large dose of radioactive amine possibly limits the interpretation of the results by the perturbation of steady-state conditions in catecholamine as well as noncatecholamine neurons. Nevertheless, this limitation was deemed acceptable since the study sought only to establish relative changes in radioactivity patterns of the ventricular perfusates under several experimental conditions. In any event, it seems clear that the radioactive catecholamine and/or metabolites appearing in cerebroventricular perfusion fluid under baseline conditions in this type of experimental procedure result predominantly by release from nonspecific (noncatecholamine-containing) storage sites, even when true tracer doses of the labelled amine have been administered (see Discussion). Following the 1 hr of equilibration, the rat was placed in a light-attenuated chamber maintained in a quiet room, and the ventricles perfused via the push-pull cannula with artificial cerebrospinal fluid [14] at a rate of 20 μl/min. A description of a prototypic fractional collection chamber for recovery of samples of perfusate and the details of perfusion have been reported previously [17,20].

Samples of the perfusate were collected every 4 min during the session; each sample cup contained 0.1 ml of 5 N acetic acid at the initiation of the collection procedure. Following the collection of the fourth sample the animal was injected i.p. with 2.0 mg/kg of *d*-amphetamine sulfate, 1.5 mg/kg of 2,5-dimethoxy-4-methylamphetamine hydrochloride (DOM) or 1.0 ml/kg of NaCl. The drugs were dissolved in NaCl. Each animal received drug or NaCl on 2 separate occasions, each separated by at least 72 hr. Immediately following the completion of a perfusion session, 10 μl aliquots from each of the 12 samples were placed into counting vials containing 15 ml of counting solution (6 g of 2,5-diphenyloxazole per liter of toluene) and 0.15 ml of BBS-3 solubilizer (Beckman Instruments, Inc., Fullerton, Calif.) The ^{14}C-radioactivity was quantified in a scintillation spectrometer. Samples of perfusate were frozen until thin-layer chromatographic (TLC) analysis could be performed (2–3 days later). Ten or 20 μl aliquots from samples Number 4 (the last sample collected before i.p. injection), 8 and 12 (approximately 16 and 32 min after injection of drug or NaCl, respectively) were spotted 15 mm from the bottom of TLC plates precoated with cellulose MN300 (Brinkman Instruments, Inc., Westbury, N. Y.). The plates were developed in a mixture of 1-butanol, methanol and 1 N formic acid (60:20:20) [4]. The solvent front was allowed to travel 150 mm in an ascending chromatography tank. After development, the plate was dried using warm air and cut up into 16 equal strips, 20 mm × 10 mm beginning 5 mm below the origin and extending 5 mm beyond the solvent front. Strips were placed sequentially in counting vials containing 1 ml of methanol and stored in a dark room for at least 24 hr. Fifteen ml of counting solution were added to each vial and ^{14}C-radioactivity was counted in a scintillation spectrometer. ^{14}C-radioactivity appearing at strips having R_f values comparable to cold norepinephrine and normetanephrine was determined for each of the 3 samples. A standard ^{14}C-NE plate was developed for each experiment in order to determine the purity of the ^{14}C-NE and to establish the per cent recovery of the ^{14}C-NE on the TLC plate (40.5% ± 5.0, based on 12 observations).

After completion of the perfusion studies all chronically implanted rats were anesthetized with sodium pentobarbital i.p. (60 mg/kg) and 5 μl of black dye was injected into the ventricle via the inner cannula tube. The animals were then decapitated and brains dissected to verify that the injected dye was contained within the ventricular system. In all cases the cannula lesion appeared to terminate in the ventricular space, at times involving slight damage to the superficial layers of the underlying brain tissue (Hippocampus and Caudate Nucleus).

Determination of Brain Catecholamine Levels

Twenty-four male albino rats of the same size as those used in the perfusion studies were divided randomly into 3 equal groups. The first group was infused intraventricularly with 6-OHDA in a manner identical to that described for the pretreatment of the first group in the perfusion experiments. The second group was infused with the ascorbic acid vehicle as in the above studies, and the third group was injected i.p. with tranylcypromine, HMD and 6-OHDOPA as described above. Twenty-one days after these treatments all 24 animals were sacrificed for the fluorometric assay of forebrain norepinephrine (NE) and

dopamine (DA) as described in detail in a previous publication [11].

RESULTS

The perfusate recovered from Control rats receiving NaCl by i.p. injection showed a gradual decline of ^{14}C-radioactivity in successive samples, as has been reported previously [17,20]. The mean values of this washout curve are shown in Fig. 1, along with those of the groups pretreated with 6-OHDOPA and 6-OHDA, for Samples 6 through 12, each expressed as a percentage of Sample 5. There were no apparent differences between the 3 groups of animals in the pattern of radioactive efflux following NaCl injection, nor were there any statistical differences between the average relative or absolute values of radioactivity in successive samples. When the data were expressed as a mean percentage of ^{14}C-radioactivity in all samples subsequent to the injection (Samples 6–12) relative to Sample 5 (the first sample after the injection), no statistical differences between groups were noted (Table 1).

There was a prominent efflux of ^{14}C-radioactivity in the perfusate 8–20 min following i.p. injection of 2.0 mg/kg *d*-amphetamine to Control animals (circles, Fig. 2). Although the efflux of ^{14}C-radioactivity observed in Samples 6 through 12 from animals pretreated with 6-OHDOPA (squares, Fig. 2) was lower than that obtained from Control animals, the mean values of the respective samples were not statistically different. The profile of radioactivity observed in animals treated with 6-OHDA showed no indication of an amphetamine-induced efflux. The mean percentage values in Samples 7 and 8 for this latter group were statistically different from corresponding means of Control animals (closed triangles, Fig. 2). Furthermore, an analysis of the average total radioactivity (average of Samples 6 through 12 relative to 5; Table 1) indicated that *d*-amphetamine increased significantly the mean radioactivity in the perfusates of Control animals (92.6% of Sample 5) as compared to the NaCl mean of this group (65.1% of Sample 5). Although *d*-amphetamine did not increase significantly the mean radioactivity in the perfusate of animals pretreated with 6-OHDOPA (83.5%) relative to this group's NaCl mean (62.7%), the mean value

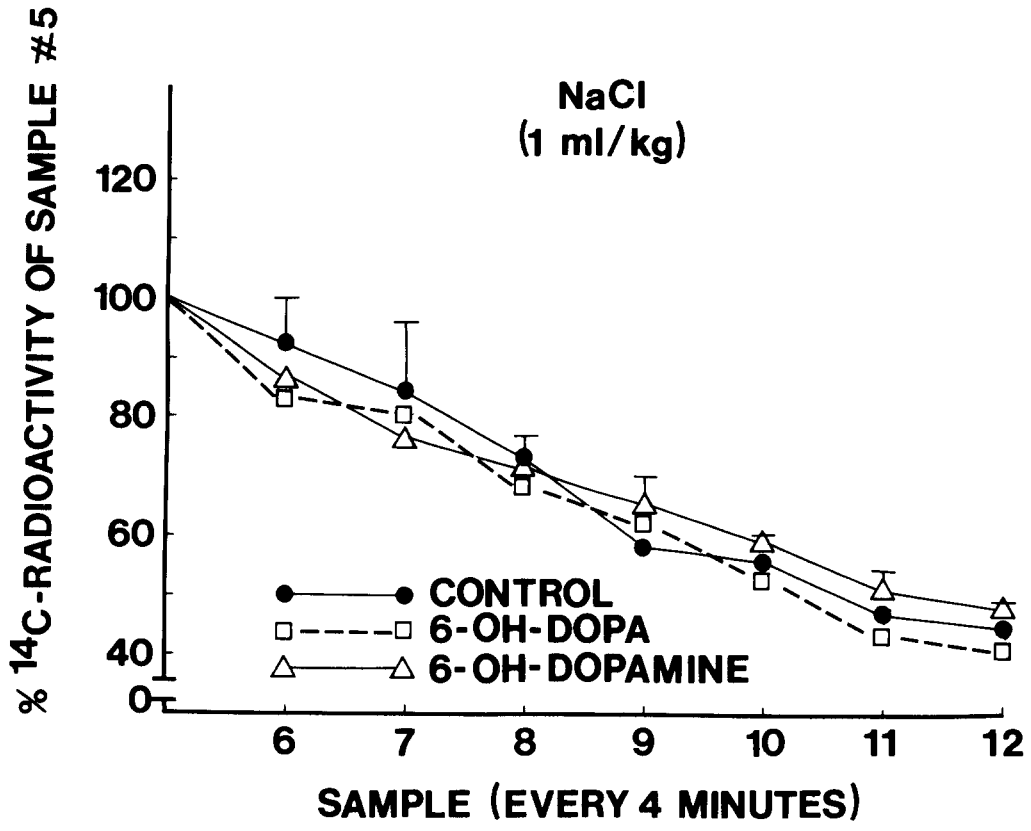

FIG. 1. Release of ^{14}C-radioactivity from ^{14}C-NE into ventricular perfusate following i.p. injection of isotonic saline (NaCl; 1 ml/kg). Injections were i.p. approximately 16 min after initiation of sample collection (after Sample 4). The data are expressed as a mean percentage ± 1 S.D. of radioactivity compared to Sample 5. The mean counts per minute (cpm) ± S.D. in Sample 5 based on 2 observations for each of 3 rats was 630 ± 239, 428 ± 162 and 438 ± 208 for the Control, 6-OHDOPA and 6-OHDA groups, respectively. The open characters (squares and triangles) for the 6-OHDOPA and 6-OHDA groups indicate no statistical difference between means of corresponding values for treated and Control animals.

TABLE 1

EFFECTS OF 6-HYDROXYDOPAMINE AND 6-HYDROXYDOPA ON THE EFFLUX OF ^{14}C-RADIOACTIVITY RECOVERED IN VENTRICULAR PERFUSATE

	Average % ^{14}C-Radioactivity of Sample 5 ± S.D.*		
	NaCl (1 ml/kg)	*d*-Amphetamine (2.0 mg/kg)	DOM (1.5 mg/kg)
Control	65.1% ± 8.6 (630 cpm ± 239)	92.6% ± 21.1‡ (683 cpm ± 564)	114.6% ± 20.2‡ (577 cpm ± 256)
6-OHDA	65.3% ± 5.5 (438 cpm ± 208)	70.4% ± 8.1† (416 cpm ± 271)	67.1% ± 11.6† (553 cpm ± 178)
6-OHDOPA	62.7% ± 4.5 (428 cpm ± 162)	83.5% ± 12.7‡ (369 cpm ± 111)	85.9% ± 11.1†‡ (774 cpm ± 218)

*Each value is the mean percentage ± S.D. of ^{14}C-radioactivity in all samples subsequent to the injection relative to sample number 5. Three rats were given each treatment twice (6 observations per mean).

†Statistically different from the mean of the Control group given the same treatment (Mann-Whitney U-test, $p<0.05$).

‡Statistically different from the mean of the NaCl treatment of the same group (Mann Whitney U-test, $p<0.05$).

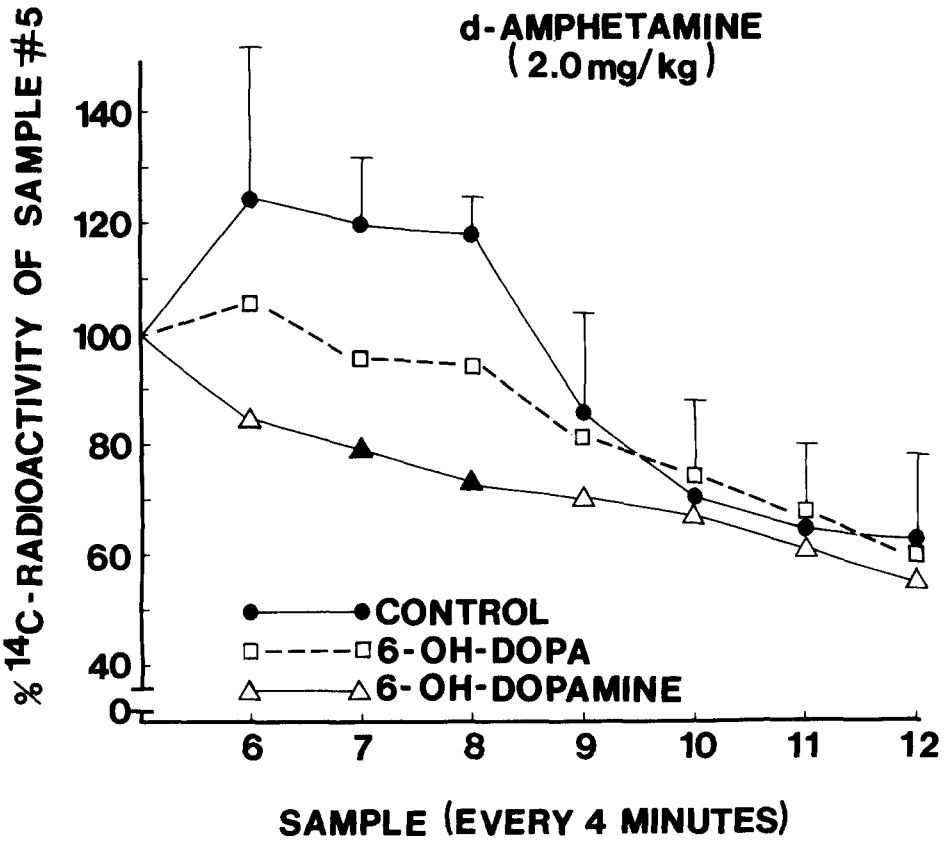

FIG. 2. Release of ^{14}C-radioactivity from ^{14}C-NE into ventricular perfusate following i.p. injection of *d*-amphetamine (2.0 mg/kg). The drug was administered after the collection of Sample 4. Values are expressed as a percentage of Sample 5 and each point is the mean of 6 values (3 rats in each group given *d*-amphetamine on 2 separate occasions). The average cpm in Sample 5 was 683 ± 564, 369 ± 111 and 416 ± 271 for the Control, 6-OHDOPA and 6-OHDA groups, respectively. Each open character for the 2 treatment groups (6-OHDA and 6-OHDOPA) indicate no statistical difference between this mean value and the corresponding Control value, while closed characters denote a statistical difference as measured by a Mann-Whitney U-test ($p<0.05$).

of this group after amphetamine was not statistically different than that of the Control group (92.6%). In rats pretreated with 6-OHDA and injected with *d*-amphetamine, the mean amount of radioactivity in Samples 6 through 12 relative to Sample 5 (70.4%) was not different than the mean following injection of NaCl to this group (65.3%), but was statistically lower than the mean radioactivity of Control animals administered *d*-amphetamine (92.6%). The average counts per minute (cpm) in Sample 5 from Control rats was not statistically different from the average cpm observed in the fifth sample from the 6-OHDA and 6-OHDOPA groups.

The i.p. injection of DOM (1.5 mg/kg) to Control animals resulted in an increased efflux of ^{14}C-radioactivity in the perfusate similar to that produced by *d*-amphetamine in these animals (circles, Fig. 3). The mean radioactivity in the perfusate in Samples 6 through 12 relative to Sample 5 for this group following DOM (114.6%, Table 1) was significantly greater than the mean NaCl value (65.1%). Animals pretreated with 6-OHDA did not show increased levels of ^{14}C-radioactivity in response to DOM. The relative amounts of radioactivity in Samples 7 through 10 (closed triangles, Fig. 3) in the 6-OHDA group were statistically lower than the corresponding values of the Control group following injection of DOM. In addition, the mean radioactivity in the perfusate (Table 1) following administration

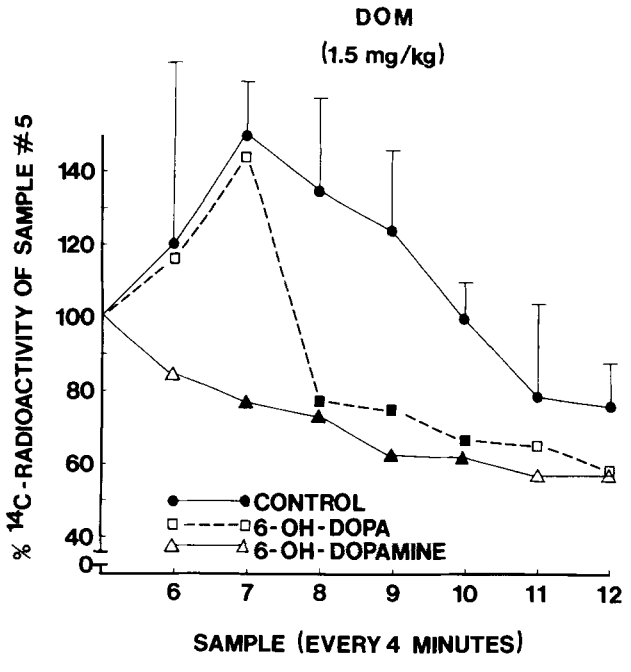

FIG. 3. Release of ^{14}C-radioactivity from ^{14}C-NE into ventricular perfusate following i.p. injection of DOM (1.5 mg/kg) immediately after collection of the fourth sample. The radioactivity measured in Samples 6 through 12 is plotted relative to Sample 5. The vertical lines indicate + 1 S.D. of the mean for Control animals and each point is the mean of 3 animals, 2 observations per animal. The average cpm ± S.D. in Sample 5 for the Control, 6-OHDOPA and 6-OHDA groups were 577 ± 256, 774 ± 218 and 553 ± 178, respectively. Statistical differences between the means of 6-OHDOPA or 6-OHDA treated animals and Control animals are denoted by the closed characters (Mann-Whitney U-test, $p<0.05$).

of DOM to this group (67.1% of Sample 5) was not statistically different from the NaCl value of this group (65.3%), but was significantly lower than the mean amount of radioactivity observed in the Control animals following DOM (114.6% of Sample 5). The most intriguing results following administration of DOM were noted in the temporal pattern of radioactive efflux from the animals pretreated with 6-OHDOPA (squares in Fig. 3). Samples 6 and 7 of this curve were not significantly different from the corresponding values observed in Control animals. However, Samples 8, 9 and 10 showed a dramatic drop of radioactivity as compared to Sample 7. The mean values of Samples 8 through 10 were statistically lower than the corresponding mean of the Control rats. As was the case when *d*-amphetamine was administered to the 6-OHDOPA group, DOM tended to increase the mean amount of radioactivity in the perfusate (85.9%). This value was statistically different from the NaCl value of this group (62.7%) and the DOM value of the Control group (114.6%). As in the other experiments, there were no significant differences between groups for the absolute amount of ^{14}C-radioactivity counted in Sample 5.

Table 2 summarizes the data obtained from the chromatographic separation of ^{14}C-radioactivity in the perfusate. The absolute number of cpm attributable to NE in Sample 4 ranged from a mean of 109 to 397 cpm and constituted 26 to 45% of the total ^{14}C-radioactivity on the TLC plate. There were no statistical differences between the mean cpm from NE in any of the preinjection samples. The ^{14}C-radioactivity attributable to NM in Sample 4 ranged from 20–82 counts, which represented 6–8% of the ^{14}C-radioactivity on the TLC plate. Only in one case, the NM in the preinjection sample taken before *d*-amphetamine administration to 6-OHDA rats, was the NM cpm statistically different from that of the NaCl Control for that group. Approximately 60% of the radioactivity on the TLC plate could not be attributed to NE or NM, with about 20% of the cpm remaining at the origin.

Following the administration of NaCl to Control rats, the ^{14}C-radioactivity attributable to NE or NM declined in Samples 8 and 12, relative to Sample 4. A similar relative decline in the proportion of counts attributable to NE in the postinjection samples was also observed in animals pretreated with 6-OHDOPA and 6-OHDA. The i.p. administration of *d*-amphetamine or DOM to Control animals produced significant increases in the proportion of counts attributable to NE and NM in Samples 8 and 12, as compared to corresponding proportions following NaCl administration. These data suggest that the increased efflux observed in the perfusate following injection of *d*-amphetamine or DOM is due, in part, to an increased release an/or a blocked reuptake of catecholamines. Slight but nonsignificant increases in the proportion of counts attributable to NE were observed in Sample 8 following *d*-amphetamine or DOM administration to 6-OHDOPA pretreated rats. Consistent drug-induced alterations in the disposition of ^{14}C-radioactivity in samples of perfusate recovered from 6-OHDA pretreated animals were not observed.

Table 3 indicates the levels of brain CA in the rats receiving identical pretreatments to those of the perfused animals and sacrificed for biochemical analyses. These results show that the 6-OHDA pretreated and implanted rats would have had reduced brain levels of norepinephrine (15% of control) and dopamine (44% of control) at the time of perfusion studies. The implanted and perfused

TABLE 2

THE EFFECTS OF 6-HYDROXYDOPAMINE AND 6-HYDROXYDOPA ON THE DRUG INDUCED APPEARANCE OF ^{14}C-NOREPINEPHRINE AND ^{14}C-NORMETANEPHRINE IN THE PERFUSATE

Group and Treatment	Mean cpm ± S.D. on TLC Plates with R_f Values for NE	NM	Mean % ^{14}C-NE and ^{14}C-NM of Sample 4 ± S.D.* Sample 8 NE	NM	Sample 12 NE	NM
Control						
NaCl	205 ± 42 (33% ± 6)†	50 ± 4 (8% ± 1.0)	54% ± 15	42% ± 13	38% ± 9	30% ± 11
DOM	210 ± 138 (34% ± 10)	42 ± 28 (7% ± 2.0)	166% ± 25‡	188% ± 12‡	81% ± 24‡	75% ± 31‡
d-Amphetamine	164 ± 62 (32% ± 5)	36 ± 8 (7% ± 0.4)	138% ± 15‡	151% ± 34‡	65% ± 14‡	72% ± 22‡
6-OHDA						
NaCl	109 ± 15 (29% ± 2)	28 ± 10 (7% ± 2.0)	43% ± 18	47% ± 13	33% ± 14	43% ± 17
DOM	169 ± 53 (28% ± 2)	39 ± 12 (7% ± 1.0)	64% ± 4	65% ± 7	41% ± 3	45% ± 12
d-Amphetamine	141 ± 38 (26% ± 6)	44 ± 21 (7% ± 1.0)	65% ± 13	62% ± 14	46% ± 16	53% ± 27
6-OHDOPA						
NaCl	312 ± 172 (45% ± 21)	37 ± 13 (6% ± 1.0)	37% ± 18	60% ± 4	30% ± 17	54% ± 10
DOM	397 ± 148 (42% ± 5)	82 ± 52 (8% ± 3.0)	48% ± 20	59% ± 25	32% ± 25	46% ± 16
d-Amphetamine	201 ± 91 (34% ± 10)	20 ± 18‡ (8% ± 1.0)	34% ± 15	53% ± 40	25% ± 11	43% ± 21

*Each value is the mean of 3 animals.

†Average percentage of total counts per minute (cpm) on the TLC plate.

‡Differs statistically from the mean value of the corresponding NaCl control group (matched pair *t*-test, $p<0.05$, for cpm; or Mann-Whitney U-test, $p<0.05$, for percentage values).

TABLE 3

THE EFFECTS OF 6-HYDROXYDOPAMINE AND 6-HYDROXYDOPA ON THE CONCENTRATION OF FOREBRAIN NOREPINEPHRINE AND DOPAMINE

Group	Mean Brain Amine Concentration (μg/g) ± S.D.* Norepinephrine	% of Control	Dopamine	% of Control
Control	0.48 ± 0.06	—	0.95 ± 0.15	—
6-OHDA	0.07 ± 0.01†	15	0.42 ± 0.02†	44
6-OHDOPA	0.25 ± 0.06†	52	1.04 ± 0.12	109

*Each value is the mean ± S.D. concentration (μg/g) of forebrain norepinephrine (NE) or dopamine (DA). There were 8 rats in each treated and Control group.

†Statistically different from corresponding mean concentration of the Control group (*t*-test, $p<0.05$).

group pretreated with 6-OHDOPA, on the other hand, would have had some reduction in brain norepinephrine (52% of control), but not dopamine (109% of control).

DISCUSSION

The results of these experiments are in accord with other studies indicating that *d*-amphetamine acts to release and/or block the reuptake of brain CA [20,24]. DOM appears to produce similar effects on brain CA as *d*-amphetamine, acting to increase the proportion of counts in the perfusate attributable to NE and NM. However, some differences in the neurochemical effects of the two agents were observed. The rapid efflux produced by DOM in 6-OHDOPA rats in early sample periods, with attenuated release in later sample periods, differed from the gradual increase of radioactivity in the perfusate over all samples following administration of *d*-amphetamine. The patterns of efflux in 6-OHDOPA rats are most likely related to the unaffected dopaminergic nerve endings in the caudate nucleus. This suggests that DOM affects CA release in two temporal phases: initial release from dopaminergic terminals followed by a secondary release from noradrenergic nerve endings.

The amphetamine-like effect of DOM on CA release is in general accord with other reports that some doses of DOM produce amphetamine-like effects on schedule-controlled behavior of rats [2, 10, 21]. Our data are supported generally by a previous report [9] that DOM increases the incorporation of tyrosine into NE. However, the doses of DOM used by Leonard [9] to obtain neurochemical effects (20–60 mg/kg) are very large compared to that used in the present study (1.5 mg/kg). In our hands, 20 mg/kg of R-DOM, the active isomer of DOM, injected i.p., produces convulsions, tremors, and death in about 50% of the animals (unpublished observation). Thus, the neurochemical effects reported by Leonard may have been associated with nonspecific stress. Recent experiments by one of us (H.A.T.) concerning the neurochemical effects of R-DOM have indicated that 2.5 to 5.0 mg/kg of this agent increases the turnover rate of dopamine, and to a lesser extent that of NE, in diencephalic and limbic forebrain areas [22]. In another study concerning the effects of hallucinogenic drugs on the metabolism of rat brain catecholamines [18], psilocybin reportedly reduced labelled norepinephrine and increased labelled normetanephrine from intracisternally administered ^{3}H-NE. These indications of extraneuronal release of NE by psilocybin were prominent up to 4 hours after injection. On the other hand, mescaline had a biphasic effect on brain NE, producing an apparent intraneuronal release of NE initially, followed by the extraneuronal release of NE later. LSD had no consistent effects on brain NE metabolism except at very high doses. These data are in accord with an earlier report in which it was observed that LSD and mescaline were ineffective in producing an efflux into ventricular perfusate from pulse-labelled stores of CA [20]. Thus, our results with DOM and those of Stolk *et al.* [18] with psilocybin indicate that these hallucinogens differ from other psychotomimetics such as mescaline and LSD in affecting brain metabolism of CA.

Differences in the manner in which psychotomimetics affect brain serotonin (5-HT) metabolism have also been reported. For example, some doses of phenethylamine derivatives such as mescaline and DOM reportedly increase concentrations of both 5-HT and its acid metabolite 5-hydroxyindole-acetic acid (5-HIAA). On the other hand, of the doses investigated indolalkylamines such as LSD and psilocybin increase brain concentrations of 5-HT, while decreasing 5-HIAA [5]. Thus, there does not appear to be a common underlying alteration in the metabolism of whole brain NE or 5-HT that can be identified as the mechanism of hallucinogenic drug action [18,20]. Since changes in brain amine metabolism are believed to be involved in the mechanism of action of psychoactive drugs [19], complex alterations in the balance of facilitatory and inhibitory neural influences may be involved in the mechanism of action of the hallucinogenic drugs [1].

The unilateral infusion of 6-OHDA into the lateral ventricles blocked the overall increase in efflux of radioactivity into the perfusate following *d*-amphetamine or DOM, suggesting that the efflux was dependent upon functional catecholaminergic nerve terminals. An alternative explanation is that the 6-OHDA blocked drug-induced efflux of radioactivity because of non-specific destruction of tissue surrounding the ventricle [13]. However, we attempted to control for this possibility by infusing the 6-OHDA slowly into the ventricles (10 μg/μl 6-OHDA infused over three minutes at a rate of 8.4 μl/min). There were no differences in the actual radioactive counts (cpm) between Control rats given NaCl and 6-OHDA pretreated animals administered NaCl, *d*-amphetamine, or DOM. If 6-OHDA pretreated rats suffered nonspecific neuronal damage, the perfusate should have contained larger amounts of radioactivity than for Control animals, since nonspecific destruction of ependymal tissue or other non-catecholaminergic neural tissue should prevent the removal of the infused ^{14}C-NE from the ventricles. The greatest proportion of labelled catecholamine injected into the lateral ventricle appears to be taken up and stored in catecholamine nerve terminals of the caudate nucleus [24] (C. C. Chiueh, personal communication). Thus, it seems paradoxical that animals in this study that were pretreated with 6-OHDA were subsequently found to exhibit the same rate of efflux of ^{14}C-NE into the perfusate, under baseline conditions, as was observed for control rats. One may suppose that the administration of nontracer doses of labelled NE could be implicated. However, the kinetics of the washout noted in these experiments are compatible with those of previous reports of studies employing this procedure, but in which tracer doses of ^{3}H-NE were administered [17,20]. Furthermore, the results of Von Voigtlander and Moore [24] indicated that the background efflux of ^{3}H-DA into ventricular perfusates in acute cat preparations was derived primarily from nonspecific storage sites. Cats that had received a tracer dose of the amine were subjected to cerebroventricular perfusion for over two hours, at which time the rate of efflux of ^{3}H-DA became relatively constant. Animals that had been lesioned in the nigrostriatal tract some weeks previously were found to release about 80% as much radioactivity as was obtained from Control cats. Yet, the chronically-lesioned animals had less than 10% of the amounts of endogenous and radioactive DA in the caudate nucleus as was observed in Control brains. Therefore, the authors concluded that the background efflux of ^{3}H-DA originated predominantly from nonspecific sites.

Blockade of drug-induced efflux of radioactivity of 6-OHDA appears contradictory to other reports indicating that intraventricular 6-OHDA does not interfere with *d*-amphetamine-induced disruption of food-reinforced

operant behavior [12] or increases in motor activity [3]. In the present investigation, brain amine determinations indicated substantial amounts of DA in the forebrains of the rats treated with 6-OHDA. Thus, an attenuation, rather than a blockade of drug-induced efflux of radioactivity would be expected. However, the unilateral infusion of 6-OHDA into or near the dopaminergic caudate nucleus may have resulted in more pronounced destruction of nerve terminals in the tissue surrounding the cannula. The NE terminals critical to the release of radioactivity into the perfusate may be those in the hippocampus and septum, since these structures are in close proximity to the push-pull cannula. Thus, little or no increase in radioactivity in the efflux over background levels would be detected in spite of CA stores available for release in other regions of the brain. It may be noted that, although 6-OHDA appeared to block the unilateral neurochemical response to *d*-amphetamine and DOM, there were indications of ongoing catecholaminegic activity elsewhere in the central nervous system. For example, gross behavioral observation of our animals following the administration of either drug indicated increased motor activity and a noticeable turning behavior ipsilateral to the infusion-perfusion side. Amphetamine-induced turning to the 6-OHDA lesioned side has been regarded as an expression of an imbalance in nervous activity in the bilateral nigrostiatal dopamine system [23].

Pretreatment with 6-OHDOPA i.p. in conjunction with a peripheral decarboxylase inhibitor (HMD) and a monoamine oxidase inhibitor (tranylcypromine) appeared to reduce the efflux of radioactivity into the perfusate following *d*-amphetamine or DOM. These findings also suggest that at the doses investigated, these two drugs affect the activity of catecholaminergic neurons in a similar manner. The degree of attenuation by 6-OHDOPA was somewhat surprising, assuming ^{14}C-NE is taken up and released from dopaminergic as well as noradrenergic nerve terminals and that 6-OHDOPA had little effect on brain DA levels. Although 6-OHDOPA reportedly does not affect the uptake of DA in vitro [8], it is possible that 6-OHDOPA alters uptake, storage or release functions of dopaminergic nerve terminals in vivo. Further work obviously is needed to clarify these issues.

REFERENCES

1. Barchas, J. D. and D. X. Freedman. Brain amines: Response to physiological stress. *Biochem. Pharmac.* **12:** 1232–1235, 1963.
2. Beaton, J. M., J. R. Smythies, F. Benington and R. D. Morin. The behavioral effects of 2,5-dimethoxy-4-methylamphetamine (DOM) in rats. *Communs Behav. Biol.*. **3:** 81–84, 1969.
3. Fibiger, H. C., H. P. Fibiger and A. P. Zis. Attenuation of amphetamine-induced motor stimulation and stereotypy by 6-hydroxydopamine in the rat. *Br. J. Pharmac.* **47:** 683–692, 1973.
4. Fleming, R. M. and W. G. Clark. Quantitative thin-layer chromatographic estimation of labelled dopamine and norepinephrine, their precursors and metabolites. *J. Chromat.* **52:** 305–312, 1970.
5. Freedman, D. X., R. Gottlieb and R. A. Lovell. Psychotomimetic drugs and brain 5-hydroxytryptamine metabolism. *Biochem. Pharmac.* **19:** 1181–1188, 1970.
6. Idänpaan-Heikkila, J. E., W. M. McIsaac, B. T. Ho, G. E. Fritchie and L. W. Tansey. Relation of pharmacological and behavioral effects of a hallucinogenic amphetamine to distribution in cat brain. *Science* **164:** 1085–1087, 1969.
7. Idänpaan-Heikkila, J. E. and W. M. McIsaac. 2,5-dimethoxy-4-methyl-amphetamine: Tissue distribution and neurochemical action. *Biochem. Pharmac.* **19:** 935–937, 1970.
8. Jonsson, G. and C. Sachs. Pharmacological modifications of the 6-hydroxydopa induced degeneration of central noradrenaline neurons. *Biochem. Pharmac.* **22:** 1709–1716, 1973.
9. Leonard, B. E. Some effects of the hallucinogenic drug 2,5-dimethoxy-4-methyl-amphetamine on the metabolism of biogenic amines in the rat brain. *Psychopharmacologia* **32:** 33–49, 1973.
10. Marquis, W. J., H. A. Tilson and R. H. Rech. Effects of amphetamine, LSD, Psilocybin and DOM on schedule-controlled behavior in the rat. *Fedn Proc.* **32:** 818, 1973 (Abstract).
11. Moore, K. E. and R. H. Rech. Antagonism by monoamine oxidase inhibitors of α-methyltyrosine-induced catecholamine depletion and behavioral depression. *J. Pharm. exp. Ther.* **156:** 70–75, 1967.
12. Peterson, D. W. and S. B. Sparber. Increased fixed ratio response rates following norepinephrine depletion by 6-hydroxydopamine. *Fedn Proc.* **32:** 753, 1973 (Abstract).
13. Poirier, J., P. Langelier, A. Roberge, R. Boucher and A. Kitsikis. Non-specific histopathological changes induced by the intracerebral injection of 6-hydroxydopamine (6-OH-DA). *J. neurol. Sci.* **16:** 401–416, 1972.
14. Robinson, R. J., R. W. Cutler, A. V. Lorenzo and C. F. Barlow. Transport of sulfate, thiosulfate and iodide by choroid plexus in vitro. *J. Neurochem.* **15:** 1169–1179, 1968.
15. Shulgin, A. T., T. Sargent and C. Naranjo. Structure-activity relationships of one-ring psychotomimetics. *Nature* **221:** 537–541, 1969.
16. Snyder, S. H., H. Weingartner and L. Faillace. DOET (2,5-dimethoxy-4-ethylamphetamine) and DOM (STP) (2,5-dimethoxy-4-methylamphetamine), new psychotropic agents: Their effects in man. In: *Psychotomimetic Drugs*, edited by D. H. Efron. New York: Raven Press, 1970, pp. 247–263.
17. Sparber, S. B. and H. A. Tilson. Schedule-controlled and drug induced release of norepinephrine -7-^{3}H into the lateral ventricle of rats. *Neuropharmac.* **11:** 453–464, 1972.
18. Stolk, J. M., J. D. Barchas, M. Goldstein, W. Boggan and D. X. Freedman. A comparison of psychotomimetic drug effects on rat brain norepinephrine metabolism. *J. Pharmac. exp. Ther.* **189:** 42–50, 1974.
19. Sulser, F. and E. Sanders-Bush. Effect of drugs on amines in the CNS. *Ann. Rev. Pharmac.* **11:** 209–230, 1971.
20. Tilson, H. A. and S. B. Sparber. Studies on the concurrent behavioral and neurochemical effects of psychoactive drugs using the push-pull cannula. *J. Pharmac. exp. Ther.* **181:** 387–398, 1972.
21. Tilson, H. A., W. J. Marquis and R. H. Rech. Effects of *d*-amphetamine, 2,5-dimethoxy-4-methylamphetamine (DOM), and psilocybin on fixed-interval responding in the rat. *Proc. Am. Psychol. Ass.*, in press, 1973.
22. Tilson, H. A., R. L. Cavanagh and T. G. Baker. Neuropharmacological analysis of R(-)-2,5-dimethoxy-4-methylamphetamine. *Pharmacologist* **16:** 205, 1974.
23. Ungerstedt, U. and G. W. Arbuthnott. Quantitative recording of rotational behavior in rats after 6-hydroxydopamine lesions of the nigro-striatal dopamine system. *Brain Res.* **24:** 485–493, 1970.
24. Von Voigtlander, P. F. and K. E. Moore. Involvement of nigro-striatal neurons in the in vivo release of dopamine by amphetamine, amantadine and tyramine. *J. Pharmac. exp. Ther.* **184:** 542–552, 1973.
25. Wallach, M. B., E. Friedman and S. Gershon. 2,5-dimethoxy-4-methylamphetamine (DOM), a neuropharmacological examination. *J. Pharmac. exp. Ther.* **182:** 145–154, 1972.