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A Comparative Study of the Effects of Prostaglandins and d-Amphetamine on the Metabolism of ^{3}H-Dopamine Continuously Presented to Rat Brain *In Vivo*[1]

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NIELSEN, J. A. AND S. B. SPARBER. *A comparative study of the effects of prostaglandins and d-amphetamine on the metabolism of ^{3}H-dopamine continuously presented to rat brain* in vivo. PHARMACOL BIOCHEM BEHAV 21(4) 583–589, 1984.—Unanesthetized rats with chronic indwelling cannulas, engaged in food reinforced operant behavior, were infused intracerebroventricularly with a solution containing a trace concentration of ^{3}H-dopamine (^{3}H-DA) with or without prostaglandins (PGs). Approximately 45 minutes after the infusion was started, the procedure was changed to a push-pull perfusion. Perfusate from the ventricles contained significant quantities of the ^{3}H-DA metabolites ^{3}H-3,4-dihydroxyphenylacetic acid (^{3}H-DOPAC), ^{3}H-3-methoxy-4-hydroxyphenylacetic acid (^{3}H-homovanillic acid, ^{3}H-HVA), ^{3}H-3-methoxytyramine (^{3}H-3-MT), and the ^{3}H-noradrenaline (^{3}H-NA) metabolite ^{3}H-3-methoxy-4-hydroxyphenylethyleneglycol (^{3}H-MHPG). The presence of $PGF_{2\alpha}$ decreased the amount of ^{3}H-DOPAC, ^{3}H-HVA, and ^{3}H-3-MT in perfusate, while PGE_1 had the opposite effects. d-Amphetamine (0.5 mg/kg, IP) affected the recovery of these metabolites from perfusate in a manner similar to $PGF_{2\alpha}$ and opposite to PGE_1. $PGF_{2\alpha}$ and the highest (seizure-inducing) dose of PGE_1 significantly decreased, while d-amphetamine significantly increased, the quantity of ^{3}H-MHPG in perfusate. Therefore, PGs affect central dopaminergic and noradrenergic activity *in vivo*, as reflected by changes in their metabolic profiles, and may play a role in the response of the central nervous system to drugs which act through catecholaminergic mechanisms.

Prostaglandins d-Amphetamine ^{3}H-dopamine ^{3}H-noradrenaline

STUDIES on the influence of various PGs on catecholaminergic neurotransmission in the periphery indicate that PGs of the F series (PGF) enhance sympathetic neurotransmission and those of the E series (PGEs) attenuate sympathetic neurotransmission (for review see [3]), although there is some controversy about the generalizability of these findings [7,21]. In the central nervous system (CNS), studies of the influence of the PGs on activity of units which receive their input from noradrenergic (NA) cells located in the locus coeruleus indicate that PGEs block or attenuate the action of NA on these cells [11] or NA presumably released as a result of stimulation of the locus coeruleus [10,20].

The studies described below were designed to further understand the relationship between PGs and catecholamines in the CNS. Originally $PGF_{2\alpha}$ and d-amphetamine were found to have similar effects on dopamine (DA) and NA neurons [15]. The present study expands upon these findings to include several concentrations of $PGF_{2\alpha}$ and PGE_1, as well as comparing and contrasting the effects of PGE_1 and $PGF_{2\alpha}$ with those of d-amphetamine alone or in combination with the PGs.

Our data indicate that PGE_1 and $PGF_{2\alpha}$ have dose-dependent, dissimilar effects upon the metabolism of ^{3}H-DA by rat brain, *in vivo*, and that they differentially interact with the action of d-amphetamine upon the metabolism (this paper) and behavioral and thermogenic action of d-amphetamine and vice versa (companion paper), suggesting that drugs which modify prostaglandin synthesis in the CNS may profoundly influence the pharmacological action of sympathomimetic agents or agents whose action is in turn influenced by catecholaminergic neurotransmission.

METHOD

Five drug-naive, mature, male Long-Evans rats (Simon-

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sen, Gilroy, CA) were housed individually in a temperature (25°C) and humidity (50%) controlled room on a 12-hour light cycle. Food and tap water were available ad lib during the study. The rats were food deprived to approximately 80% of their free-feeding weights (400–450 g) and shaped to lever press on a fixed-interval 75-second schedule for food reinforcement.

The five rats used in this neurochemical experiment were also used in concurrent behavioral and body temperature experiments. The effects of PGs and/or d-amphetamine upon the latter two parameters will be discussed in the companion paper.

Cannula Implantation

The rats were implanted with Gaddum [9]-type push-pull cannulas with cannula tips in the right lateral ventricles. The cannulas were made by modifying stainless steel infusion cannulas (D. A. Kopf, Tujunga, CA). The cannula bases, caps and styluses were constructed and implanted as described previously in detail [15]. One rat did not complete all of the experiments because its cannula fell out. The data collected from this rat was similar to that described below for the other rats. However, since the fifth rat did not complete the entire experiment, the data presented are for 4 rats.

Perfusion Procedure

Ten days after cannula implantation, 3 μl of a sterile saline solution containing approximately 0.27 μCi of ^{45}Ca (New England Nuclear, Boston, MA) were pulled into the end of the infusion tube. Then the animals were placed in operant chambers and the push and pull tubes were connected with silicone tubing to the infusion and perfusion pumps (Compact Infusion Pump, Harvard Apparatus, Millis, MA, to push fluid into the brain; and a Minipuls II, HP4, Gilson Electronics, Middleton, WI, to pull fluid from the brain). Ten minutes after the beginning of the operant behavioral session, infusion (1 μl/min) of the infusion-perfusion medium (the ^{45}Ca solution then sterile saline containing 2.3 mM $CaCl_2$ plus about 5.8 μCi or 100 ng of ^{3}H-DA) was begun and continued for 45 minutes. This was followed immediately by a 5 min perfusion (10 μl/min) of the same medium. At the conclusion of the session, the rat was disconnected from the pumps, the stylus was screwed into the cannula base, and the animals were returned to their home cage.

Perfusate was collected into preweighed polyethylene microsample tubes which contained various catecholamines and metabolites in 5 μl of 1.0 N formic acid *(vide infra)*. After the experiment, the perfusate was weighed and refrigerated overnight at 4°C. Experiments were performed at about the same time each day.

Analysis of Perfusate

The perfusate was analyzed the day after collection, as described in detail elsewhere [15]. Briefly, a 10 μl aliquot of the 50 μl perfusate was separated by the two-dimensional thin-layer chromatographic system of Fleming and Clark [8] into spots which cochromatographed with authentic DOPAC, HVA, 3-MT, MHPG, DA, NA, normetanephrine (NM), 3-methoxy-4-hydroxymandelic acid (VMA), 3,4-dihydroxymandelic acid (DHMA), 3,4-dihydroxyphenylethyleneglycol (DHPG), 3,4-dihydroxyphenylethanol (DHPET), and 3-methoxy-4-hydroxyphenylethanol (HVET). The radioactivity on these spots was determined using liquid scintillation spectrometry (LS 150, Beckman, Fullerton, CA). Blank values for perfusate samples were derived by determining the radioactivity found at the appropriate spots on control plates, where an aliquot of the infusion-perfusion medium, not exposed to the rat's brain, was analyzed as described above. Perfusion medium contained approximately 1.25×10^5 DPM in 10 μl. When spotted on TLC plates, approximately 20% of the ^{3}H was lost upon evaporation. After machine background was subtracted (20 cpm) and CPM converted to DPM, using external standard ratios and quench curve (counting efficiency for ^{3}H samples approximated 45%), all data was normalized to represent DPM/10^5 DPM nonvolatile tritium. The nonvolatile tritium (the amount of tritium available for migration after evaporation and loss of volatile tritium) is the quantity of radioactivity found in an aliquot of perfusion medium or perfusate that is not separated into the different ^{3}H-DA metabolites on the thin-layer plates. This aliquot was merely spotted in the upper right corner (diagonally opposite the spot to be separated) and exposed to solvent system vapors.

Each 10 μl aliquot of perfusion medium or perfusate actually contained about 10^5 DPM of nonvolatile tritium which was available for further separation on the TLC plate. Therefore, although normalizing the data in this manner allowed us to compare different perfusions, it in no way inflated the data. Determining exact recoveries for each of the important metabolites was not deemed necessary because we were comparing and contrasting the relative effects of exposure to peripherally administered amphetamine and/or centrally administered PGs.

The amount of ^{45}Ca in perfusate was determined by analyzing a 10 μl aliquot of perfusate using liquid scintillation spectrometry. A variable discriminator window was set to determine the quantity of radioactivity associated with ^{45}Ca. It was determined that essentially no ^{3}H was counted in this window. The data were corrected for the radioactivity (approximately 20%) associated with ^{45}Ca which was counted in the ^{3}H window. The data were also corrected for the radioactive decay of ^{45}Ca.

Experimental Manipulations

The first experiment involved the infusion-perfusion of the medium containing ^{3}H-DA and injection of d-amphetamine (0.5 mg/kg, IP); then six nondrug and three d-amphetamine replicate experiments were performed. These nine experiments were performed before, during and after the various PG experiments, in order to determine and/or control for sources of variation in the rats' behavior, temperature, and amount of ^{3}H-DA and ^{3}H-NA metabolites in brain perfusate during nondrug or d-amphetamine experiments changed systematically due to the passage of time, repeated experiments, drug interaction or other carry-over effects.

Twelve experiments were performed where PG was added to the infusion-perfusion medium, two at each concentration of $PGF_{2\alpha}$ (10, 100 and 1000 ng/μl) and PGE_1 (100, 250 and 500 ng/μl). These doses were based on our previous work [15]. In half of the experiments saline was injected; in the other half, d-amphetamine (0.5 mg/kg) was injected at the 23rd minute of the experiment (see Table 1, accompanying paper). The experiments were randomized to a certain extent, but equipment constraints necessitated the administration of the same infusion-perfusion medium to all rats on the same day.

TABLE 1

VARIABILITY BETWEEN RATS, BUT STABILITY WITHIN RATS IN THE QUANTITY OF ^{3}H-DOPAMINE METABOLITES IN RAT BRAIN PERFUSATE*

Rat	Injection	^{3}H-DOPAC	^{3}H-HVA	^{3}H-3-MT	^{3}H-MHPG
101	saline	174 ± 27	234 ± 16	92 ± 8	46 ± 7
	d-amphetamine	120 ± 6	120 ± 12	35 ± 6	96 ± 7
104	saline	101 ± 10	229 ± 19	193 ± 9	31 ± 5
	d-amphetamine	66 ± 5	111 ± 6	49 ± 5	63 ± 3
105	saline	227 ± 16	212 ± 32	126 ± 59	99 ± 5
	d-amphetamine	104 ± 6	128 ± 9	18 ± 6	206 ± 24
108	saline	399 ± 41	98 ± 11	105 ± 9	52 ± 6
	d-amphetamine	195 ± 7	50 ± 5	29 ± 3	119 ± 10

*The rats were infused with a solution containing a trace concentration of ^{3}H-dopamine for 45 minutes followed by a 5-minute perfusion with the same medium. Saline (6 experiments) or d-amphetamine (4 experiments) was injected 35 minutes before the beginning of the perfusion. Data represent the quantity of the different ^{3}H-dopamine metabolites recovered in 10 μl aliquots of the perfusate. See Table 2 for an explanation of data expression. Mean ± S.E.

Drugs, Infusion-Perfusion Medium

d-Amphetamine sulfate (K and K Laboratories, Inc., Plainview, NY) was dissolved in isotonic saline in a concentration of 0.5 mg of the base per ml and injected intraperitoneally (IP) in a volume of 1 ml/kg body weight. The infusion-perfusion medium contained sterile 0.9% saline to which tritium labelled dopamine (^{3}H-2-dopamine, 0.1 ng/μl, Sp. Act. 7.5 Ci/mM, New England Nuclear) and $CaCl_2$ (2.3 mM) had been added. During the first 3 min of infusion the solution contained $^{45}CaCl_2$ (0.2 μCi/mM, New England Nuclear). Accounting for decay, the actual ^{45}Ca injected was about 0.28 μCi. Infusion and perfusion were at rates of 1 and 10 μl/min, respectively. In some experiments, $PGF_{2\alpha}$ (10, 100 or 1000 ng/μl) or PGE_1 (100, 250 or 500 ng/μl) was added to the infusion-perfusion medium. The PGs (kindly supplied by Dr. J. Pike, The Upjohn Company, Kalamazoo, MI) were stored in absolute ethanol at −20°C. The ethanol in the PG stock solution was evaporated under nitrogen before the PG was added to the infusion-perfusion medium.

Data Analysis

Data were analyzed by a paired Student t-test, with each rat serving as its own control, to determine if d-amphetamine altered the parameters that were measured. To determine if PGs and/or d-amphetamine had effects, data were analyzed by a one-way analysis of variance, randomized block design, with replication (each subject being exposed to 8 treatment conditions) as the blocking factor. Significant differences between treatment means were determined by the Bonferroni [14] significant difference test.

RESULTS

Nondrug Experiments

Tritium-labelled compounds which cochromatographed with authentic DOPAC, HVA, 3-MT, and MHPG were recovered in quantities significantly above background. No tritium-labelled compounds which cochromatographed with authentic HVET, DHPET, DHMA, VMA, NA or NM were recovered in perfusate in quantities significantly above blank values. There was much variability between rats in the quantity of the metabolites in perfusate. However, for each rat, the quantity of each metabolite in the six nondrug experiments was consistent (Table 1). Since nondrug experiments were performed before, during and after the PG and/or d-amphetamine experiments, it was concluded that the quantity of metabolites of ^{3}H-DA in perfusate was not systematically altered by the various PG and/or d-amphetamine experiments.

d-Amphetamine

d-Amphetamine (0.5 mg/kg) caused a significant decrease in the quantities of ^{3}H-DOPAC, ^{3}H-HVA, and ^{3}H-3-MT in perfusate and a significant increase in the quantity of ^{3}H-MHPG in perfusate (Table 2). d-Amphetamine also increased the quantity of ^{45}Ca in perfusate (Fig. 1). For each rat there was little variability in the quantities of ^{3}H-DOPAC, ^{3}H-HVA, ^{3}H-3-MT and ^{3}H-MHPG recovered in perfusate in the four d-amphetamine experiments carried out at various times throughout the course of experimentation (Table 1).

Prostaglandins

Infusion of 100, 250 and 500 ng/min of PGE_1 produced convulsions in 0, 2, and 4 out of 4 rats, respectively. The convulsions occurred after about 10 μg of PGE_1 had been infused whether at a rate of 250 or 500 ng/minute.

The prostaglandins continuously presented to brain along with ^{3}H-DA produced consistent and differential effects upon the metabolic profile of DA and NA. $PGF_{2\alpha}$ reduced the amount of all three ^{3}H-DA metabolites and ^{3}H-MHPG in a concentration-dependent manner (Table 2). When PGE_1 was added to perfusion medium, an entirely different profile emerged. PGE_1 produced significant concentration-dependent increases in ^{3}H-DOPAC, ^{3}H-HVA and ^{3}H-3-MT (Table 3). Unlike $PGF_{2\alpha}$, PGE_1 had no systematic effect upon the amount of ^{3}H-MHPG in perfusate, except for the highest concentration, which caused a significant reduction of the NA metabolite (Table 3). Both PGs increased the efflux of ^{45}Ca into perfusate (Fig. 1).

TABLE 2

EFFECTS OF $PGF_{2\alpha}$ AND/OR D-AMPHETAMINE ON QUANTITIES OF ^{3}H-DOPAC, ^{3}H-HVA, ^{3}H-3-MT AND ^{3}H-MHPG IN RAT BRAIN VENTRICULAR PERFUSATE

Injection	Infusion	^{3}H-DOPAC	^{3}H-HVA	^{3}H-3-MT	^{3}H-MHPG
saline	vehicle	226 ± 64	193 ± 32	129 ± 22	57 ± 15
d-amphetamine	vehicle	121 ± 26#	101 ± 18##	32 ± 7##	122 ± 30#
saline	$PGF_{2\alpha}$ 10	114 ± 36*	87 ± 11*	58 ± 17**	29 ± 10*
saline	100	12 ± 5*	9 ± 5***	36 ± 11**	14 ± 4***
saline	1000	1 ± 1*	0 ± 0***	14 ± 4**	0 ± 0***
d-amphetamine	$PGF_{2\alpha}$ 10	58 ± 19	50 ± 9###	67 ± 27	72 ± 16#
d-amphetamine	100	3 ± 1	4 ± 4	10 ± 5###	31 ± 9##
d-amphetamine	1000	0 ± 0	4 ± 4	0 ± 0##	1 ± 1

Four rats were infused (1 μl/min) with sterile saline containing 2.3 mM $CaCl_2$ and a trace concentration of ^{3}H-DA. The infusion lasted 45 minutes and was followed by a 5-minute perfusion with the same medium. Effects of saline (1 ml/kg, IP) or d-amphetamine (0.5 mg/kg, IP) injected 10 minutes after initiating the infusion and/or $PGF_{2\alpha}$ (ng/μl/min, ICV) infused for 45 minutes. Quantities of ^{3}H-metabolites are expressed as dpm/10^5 dpm nonvolatile tritium/10 μl of perfusate. Mean ± 1 S.E. of 4 rats. *$p<0.05$ **$p<0.01$ ***$p<0.005$ compared with vehicle infusion, #$p<0.05$ ##$p<0.01$ ###$p<0.005$ compared with saline injections.

TABLE 3

EFFECTS OF PGE_1 AND/OR D-AMPHETAMINE ON QUANTITIES OF ^{3}H-DOPAC, ^{3}H-HVA, ^{3}H-3-MT AND ^{3}H-MHPG IN RAT BRAIN VENTRICULAR PERFUSATE

Injection	Infusion	^{3}H-DOPAC	^{3}H-HVA	^{3}H-3-MT	^{3}H-MHPG
saline	vehicle	226 ± 64	193 ± 32	129 ± 22	57 ± 15
d-amphetamine	vehicle	121 ± 26#	101 ± 18##	32 ± 7##	122 ± 30#
saline	PGE_1 100	812 ± 206*	603 ± 155*	183 ± 40	40 ± 9
saline	250	1099 ± 50***	908 ± 55***	352 ± 113*	63 ± 15
saline	500	2131 ± 156***	2307 ± 239***	457 ± 115*	16 ± 3*
d-amphetamine	PGE_1 100	438 ± 187#	323 ± 47#	125 ± 25	84 ± 53#
d-amphetamine	250	438 ± 88##	532 ± 44#	237 ± 55#	8 ± 5#
d-amphetamine	500	1081 ± 90##	1061 ± 48##	108 ± 29##	7 ± 3#

See Table 2 for an explanation of the experimental protocol and data presentation. *$p<0.05$ **$p<0.01$ ***$p<0.005$ compared with vehicle infusion, #$p<0.05$ ##$p<0.01$ ###$p<0.005$ compared with saline injections.

Prostaglandins and d-Amphetamine

d-Amphetamine (0.5 mg/kg) decreased the quantity of ^{3}H-DOPAC and ^{3}H-HVA in perfusate by about 50%, regardless of whether $PGF_{2\alpha}$ or PGE_1 was infused (Tables 2 and 3). The effects of d-amphetamine occurred over a 200-fold range of counts attributable to ^{3}H-DOPAC and ^{3}H-HVA in perfusate. This effect could not be demonstrated when the highest concentration of $PGF_{2\alpha}$ was infused because the ^{3}H-DOPAC and ^{3}H-HVA in perfusate were already near undetectable levels. As a consequence there was a greater reduction in ^{3}H-DOPAC and ^{3}H-HVA when d-amphetamine was injected during experiments in which $PGF_{2\alpha}$ was infused. However, when d-amphetamine was given in conjunction with PGE_1, an antagonistic effect was observed, since PGE_1 tended to increase ^{3}H-DOPAC, ^{3}H-HVA, and ^{3}H-3-MT by itself. d-Amphetamine doubled the quantity of ^{3}H-MHPG in perfusate, regardless of whether or not $PGF_{2\alpha}$ was infused (Table 2), except at the highest concentration of $PGF_{2\alpha}$, which was probably supramaximal in its capacity to lower ^{3}H-MHPG.

The lowest concentration of PGE_1 (100 ng/minute) had no effect on the amount of ^{3}H-3-MT (Table 3) in perfusate, however, it did attenuate the d-amphetamine-induced decrease in ^{3}H-3-MT in perfusate. Similarly, a concentration of PGE_1 (250 ng/minute), which itself did not alter the quantity of ^{3}H-MHPG, reversed the d-amphetamine-induced increase in ^{3}H-MHPG in perfusate (Table 3). At higher concentrations PGE_1 had effects on the recovery of ^{3}H-3-MT and ^{3}H-MHPG opposite to those of d-amphetamine.

DISCUSSION

A method which may measure changes in CNS dopaminergic and noradrenergic neurons in unanesthetized,

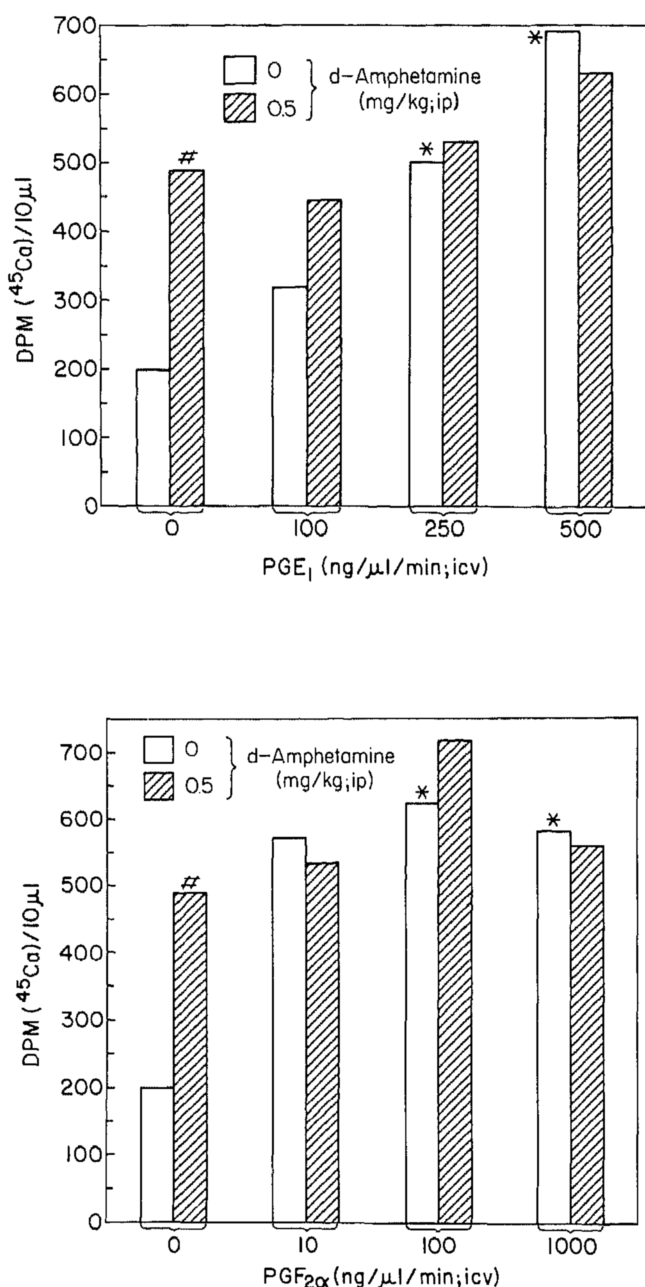

FIG. 1. PGE_1, $PGF_{2\alpha}$, and d-amphetamine increased the quantity of ^{45}Ca in rat-brain ventricular perfusate. Four rats were infused (1 μl/min) with a sterile saline solution containing 2.3 mM $CaCl_2$, a trace concentration of ^{3}H-DA, and sometimes PGE_1 or $PGF_{2\alpha}$. The first 3 μl contained a trace dose of ^{45}Ca which was completely washed out of the tubing and into the brain during the first 10 minutes of the infusion. The infusion lasted 45 minutes and was followed by a 5 minute perfusion with the same medium. Effects of saline (1 ml/kg, IP; open bars) or d-amphetamine (0.5 mg/kg, IP; cross-hatched bars) injected 10 minutes after initiating the infusion (35 to 40 minutes prior to collection of perfusate) and/or infusion of PGE_1 (100, 250, and 500 ng/μl/min; ICV, top panel) or $PGF_{2\alpha}$ (10, 100, and 1000 ng/μl/min; ICV, bottom panel) for 45 min immediately prior to collection of perfusate are shown. The data shown is the mean of 4 rats. One standard error about each mean ranged from 28 to 220 DPM (^{45}Ca)/10 μl. #$p<0.05$ compared with saline injection, *$p<0.05$ compared with vehicle infusion.

freely-moving rats was used in these experiments. Infusion of a solution containing a trace concentration of ^{3}H-DA into the lateral ventricular space of rats resulted in recovery in perfusate of ^{3}H-DOPAC, ^{3}H-HVA and, to a lesser extent, ^{3}H-3-MT and ^{3}H-MHPG. The perfusate contained metabolites of ^{3}H-DA in proportion to their concentration in rat brain tissue [2]. ^{3}H-DA also appears to have been metabolized in a manner similar to endogenous DA (for review see [19]). Therefore, our data suggest that the ^{3}H-DA in the perfusion medium was taken into neurons and metabolized in the normal manner in the absence or presence of drug treatment. One would not expect d-amphetamine to affect the ^{3}H-DA in perfusate if the ^{3}H-DA were taken into glial cells or processed in some other nonspecific way.

Amphetamine decreased the amount of ^{3}H-DA metabolites and increased ^{3}H-MHPG in perfusate. Several explanations for these results could be proposed. Most importantly these results were used to compare and contrast the effects of PGE_1 and $PGF_{2\alpha}$ with d-amphetamine.

$PGF_{2\alpha}$ and d-amphetamine had similar effects on recovery of ^{3}H-DA metabolites in perfusate. At the lower doses of $PGF_{2\alpha}$ the effects could be viewed as being additive with or occuring independent of d-amphetamine; either way these results suggest that $PGF_{2\alpha}$ and d-amphetamine are working through different mechanisms. d-Amphetamine and the highest dose of $PGF_{2\alpha}$ were not additive due perhaps to a floor effect, that is the inability to observe a decrease due to the sensitivity of the assay.

$PGF_{2\alpha}$ and d-amphetamine had opposite effects on the amount of ^{3}H-MHPG in perfusate. d-Amphetamine doubled the amount of ^{3}H-MHPG in perfusate, regardless of whether or not $PGF_{2\alpha}$ was infused, except at the highest concentration of $PGF_{2\alpha}$. Perhaps that concentration was supramaximal in terms of its effects on the amount of ^{3}H-MHPG in perfusate, and d-amphetamine therefore was not able to overcome its effect.

PGE_1 increased the recovery of metabolites of ^{3}H-DA in perfusate. The convulsions (companion paper) may have influenced these effects of PGE_1. However, these effects also occurred at a concentration of PGE_1 that did not produce convulsions. Only the highest dose of PGE_1 decreased the amount of ^{3}H-MHPG in perfusate. Therefore, it is uncertain whether this effect is directly due to the PG, or indirectly due to the convulsions which occurred at the high dose.

The convulsions were not due to a nonspecific lipid effect since infusion of a higher concentration (1000 ng/minute) of a structurally similar lipid, $PGF_{2\alpha}$, produced no behavioral evidence seizures. Further discussion of the PGE_1-induced convulsions is included in the companion paper. However, the convulsions did not appear to have any permanent effects on the parameters that were measured, since nondrug and d-amphetamine experiments performed a few days after all of the rats had experienced convulsions resulted in a temperature, behavior (companion paper), and metabolic profile similar to that observed in experiments before the convulsions.

Early studies on the involvement of PGs in CNS function and neurochemical phenomena tried to establish a link between catecholamines and PGs by infusing the biogenic amines into brain in high concentrations and looking for evidence of release of PGs. For example, Holmes [12] reported that perfusion of dogs' ventricular space with a high concentration of NA or adrenaline had no effect upon PG release. If some sort of reciprocal inhibitory relationship exists between catecholamines and PGE, for example, PGE should

have appeared in ventricular space. Our studies demonstrate an effect of exogenous PGs upon metabolism of ^{3}H-DA (metabolism which we infer is a reflection of endogenous catecholaminergic activity). Perhaps technological capabilities did not allow relatively small and/or transient changes in PG levels or their utilization to be determined in earlier work from other laboratories. Alternatively, it may be that the most important relationship between PGs and neurotransmitters in the CNS is a unidirectional one; the PGs modulating release and/or postjunctional actions of amines upon pathophysiological or pharmacological perturbation in aminergic systems in a manner different from that achieved by direct injection of catecholamines into brain.

The effect of PGEs on DA neurons is uncertain. Bergstrom and coworkers [1] found that PGE_2 attenuated the electrically evoked release of DA from slices of rat striatum. However, PGE_2 did not affect the release of DA from the cat striatum *in vivo* [24]. More recently Reimann and coworkers [16] reported that PGE_2 and $PGF_{2\alpha}$ had no effect on the basal efflux or the stimulation evoked efflux of ^{3}H-DA from striatal slices. The PGE_1, which shares almost all of PGE_2's central actions (for review, see [5]), increased ^{3}H-DA metabolites in perfusate; an effect which was attenuated by a compound which stimulates the release of DA. Since compounds in the cerebrospinal fluid are thought to originate largely from the striatum [6,13], the findings taken together would suggest that PGE_1 inhibits the release of DA from the rat striatum *in vivo*. However, the septal nucleus also borders the lateral ventricle, and PGE_2 affects DA neurons in the septum [22], therefore PGE_2 might also be inhibiting the release of DA from the rat septum *in vivo*.

The effect of PGE_1, $PGF_{2\alpha}$, and d-amphetamine on the amount of ^{45}Ca in perfusate also suggests that these compounds are all affecting neurotransmitter release mechanisms. The key event in triggering release of neurotransmitters is the movement of calcium ions from the external fluid into the cell. In our experiments both PGs and d-amphetamine increased the quantity of ^{45}Ca in perfusate, perhaps as a consequence of displacement or mobilization of bound ^{45}Ca. While the exact mechanism of this action is uncertain, it suggests that PGs, like d-amphetamine, might be affecting neurotransmitter release mechanisms.

Vijayalakshmi and coworkers [23] found that PGE_2 and $PGF_{2\alpha}$ increased the activity of monoamine oxidase in rat brain. If PGFs and PGEs were merely increasing monoamine oxidase activity, then $PGF_{2\alpha}$ and PGE_1 would not have decreased the quantities of metabolites of ^{3}H-DA and ^{3}H-NA in perfusate, respectively. Therefore, it appears that PGs have a more complex action on catecholaminergic neurotransmission than merely altering enzymatic activity.

PGE_1 might also be important in the physiological antagonism of d-amphetamine's actions. d-Amphetamine, through release of NA, may stimulate the synthesis of PGEs [17,25]. Perhaps the PGEs then act to reduce the actions of amphetamine. Support for this hypothesis comes from Sever and Trelinski [18] and Caldwell and Putman [4], who found that PG synthetase inhibitors potentiated the increase in body temperature caused by a high dose of d-amphetamine.

In summary, PGE_1 and $PGF_{2\alpha}$ altered the profile of *in vivo* metabolism of ^{3}H-DA presented to rat brain. Although the effects of $PGF_{2\alpha}$ and d-amphetamine are similar in many respects, the data suggests that $PGF_{2\alpha}$ alters the release of DA and NA in a manner unlike d-amphetamine. PGE_1 antagonized d-amphetamine's actions and d-amphetamine antagonized PGE_1-induced convulsions (companion paper), suggesting that PGE_1 inhibits the release of catecholamines from central neurons *in vivo*, and is a physiological antagonist of d-amphetamine. Since the perfusate was only collected at the end of the experiment, the PG-induced change in catecholamine neurons may reflect a compensatory, rather than the initial effect of the compounds. However, there is little doubt that PGs affect catecholamine neurons. Therefore, PGs may play a role in the response of the CNS to drugs which act through catecholaminergic mechanisms. It is also probable that many effects of such drugs can be influenced by agents which alter the synthesis, release or metabolism of the PGs.

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