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Metabolism of ^{3}H-Dopamine Continuously Perfused Through Push-Pull Cannulas in Rats' Brains: Modification by Amphetamine or Prostaglandin $F_{2\alpha}$[1]

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NIELSEN, J. A., L. H. FOSSOM AND S. B. SPARBER. *Metabolism of ^{3}H-dopamine continuously perfused through push-pull cannulas in rats' brains: Modification by amphetamine or prostaglandin $F_{2\alpha}$.* PHARMAC. BIOCHEM. BEHAV. 13(2) 235–242, 1980.—Using perfusion cannulas implanted in the lateral cerebroventricles, the metabolism of a trace concentration of ^{3}H-dopamine, continuously presented, was investigated in rats performing an operant for food reinforcement. The subjects were mature, drug-naive, male Long-Evans rats. Perfusate contained measurable quantities of ^{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 metabolite, ^{3}H-3-methoxy-4-hydroxyphenylethyleneglycol (^{3}H-MHPG). Systemic injection of d-(1.5 mg/kg) or l-(3.0 mg/kg) amphetamine resulted in decreased quantities of ^{3}H-DOPAC, ^{3}H-HVA and ^{3}H-MHPG in perfusate with a concurrent decrease in fixed-ratio 20 behavior. Addition of prostaglandin $F_{2\alpha}$ (10 ng/μl perfused at a rate of 10 μl/minute) had no effect on the rats' fixed-ratio 20 behavior or rectal temperature, but resulted in decreased quantities of ^{3}H-DOPAC, ^{3}H-HVA and ^{3}H-MHPG in perfusate. It is concluded that this methodology allowed us to monitor drug-induced changes in CNS dopaminergic and noradrenergic function in conscious rats engaged in schedule-controlled operant behavior. Furthermore, a trace concentration of $PGF_{2\alpha}$ in perfusion medium caused changes in ^{3}H-dopamine metabolism in a manner similar to that of systemically administered amphetamines.

Brain perfusion Dopamine Noradrenaline Amphetamine Prostaglandin Operant behavior

ANALYSIS of fluid withdrawn from the brain can tell us much about the dynamics of central nervous system (CNS) neurotransmission and thus may indirectly reflect changes in the activity of neurons. Several techniques have been used to withdraw fluid from the CNS. Feldberg and coworkers [4] developed a technique for perfusing the cerebral ventricles. Gaddum [11] suggested the use of the push-pull cannula for perfusion of localized brain regions. Several laboratories have used this system to study CNS neurochemistry (eg. [3,21]). One aspect of CNS neurochemistry that has been studied involves drug- and other experimentally-induced changes in quantities of putative neurotransmitter metabolites in brain perfusate [18, 19, 22]. In most studies the animals have been anesthetized, thus complicating the use of this technique for studying the CNS, since sedative-hypnotic agents have been shown to variously affect neuronal activity [13] or neurochemical parameters [2,8]. We have attempted to overcome this problem by using techniques which allow us to measure neurochemical and behavioral changes concurrently in conscious, freely-moving rats engaged in complex operant behavior [28].

This report presents a detailed description of the refined push-pull perfusion technique presently employed in our laboratory. The major thrust of this communication should be viewed as a demonstration that continuous perfusion of the ventricular space with ^{3}H-dopamine (^{3}H-DA) is one method of determining if changes in catecholaminergic activity in brain are measurable *in vivo* in conscious animals engaged in operant behavior. We will also show that low doses of d- and l-amphetamine, injected systemically, produced concurrent changes in fixed-ratio 20 behavior and reflections of changes in dopaminergic and noradrenergic neuronal function. Having established the utility of this approach, we initiated a series of studies to determine if a prostaglandin (PG), at concentrations devoid of behavioral or thermal effects, could significantly alter the way in which brain metabolizes catecholamines. The prediction that this should be the case is based primarily upon neuropharmacological studies which report

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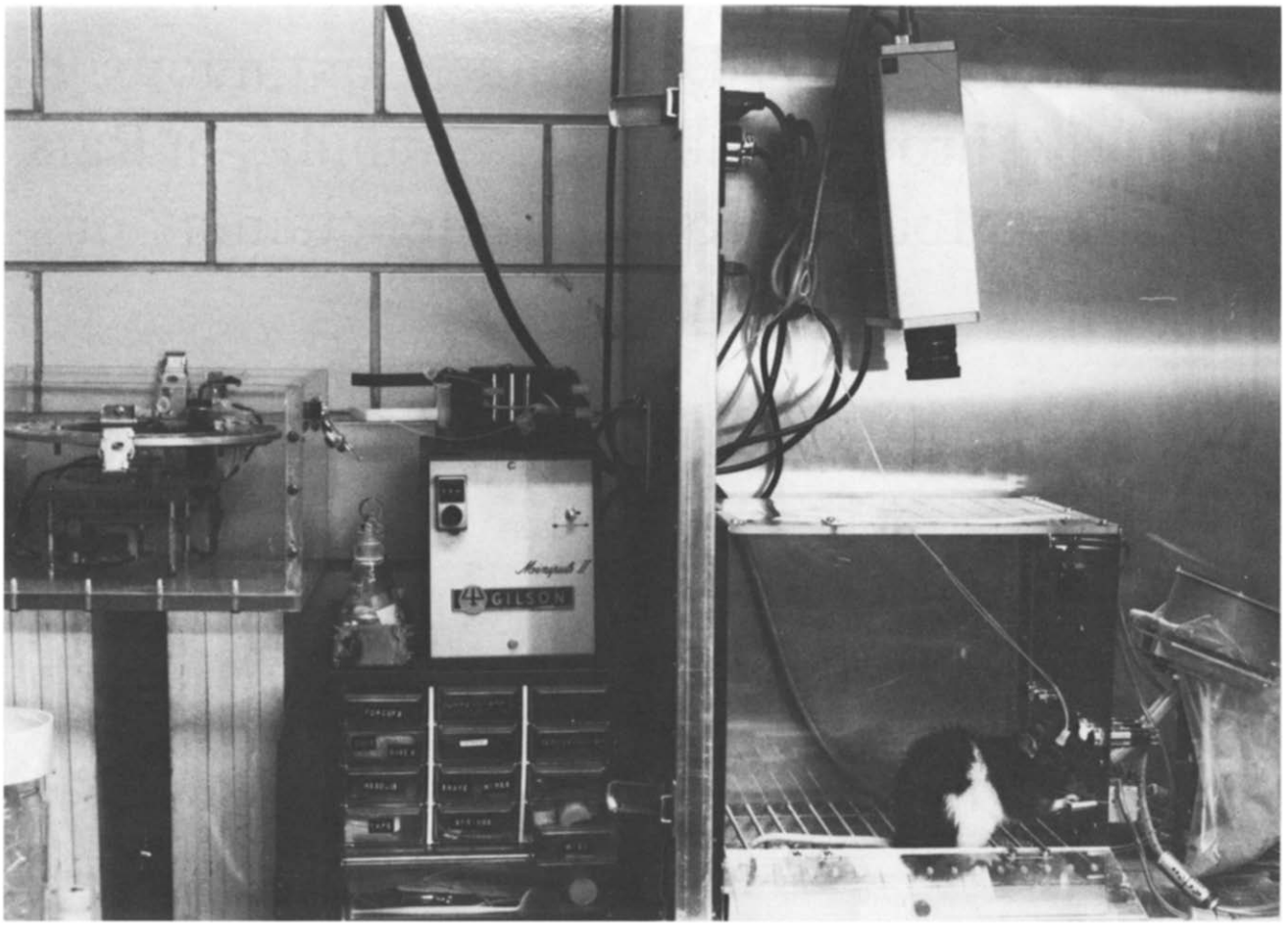

FIG. 1. A rat chronically implanted with a push-pull cannula responding for food reinforcement during a perfusion session. The custom made enclosure allows closed circuit television monitoring of behavior sessions. Adequate lengths of silastic tubing allow complete freedom of movement. Samples are collected in microsample tubes in the small fraction collector.

interactions between catecholamines and prostaglandins in restricted cell populations [14].

We have included a multivariate experimental design, examining complex behavior, ^{3}H-DA metabolism and drug action, for several reasons. Firstly, the surgical and perfusion procedure itself may result in sufficient trauma to alter CNS function, manifest as a behavioral change and/or biochemical change. Secondly, presentation of exogenous ^{3}H-DA and/or $PGF_{2\alpha}$, alone or in combination, might alter CNS function (i.e. pharmacologically relevant concentrations). Thirdly, these experiments were a prelude to more extensive ones in which the multidisciplinary approach to behavioral/biochemical analyses of drug action, which we have used successfully in the past to study psychoactive agents such as amphetamine, LSD-25, mescaline, morphine and other drugs [30,32], would be expanded to include the PG's and other substances found in the CNS (and elsewhere). In essence, we are attempting to study these agents under conditions which approximate the so-called physiological state, a condition not nearly approached so closely in studies which rely upon extirpation, slicing, chopping, homogenizing or otherwise separating neuronal tissue from the remainder of the organism.

METHOD

Operant Behavior

Eleven mature, male Long-Evans rats (Simonsen, Gilroy, CA) were caged individually in a room on a 12 hour day-night cycle. Food and tap water were available ad lib for several weeks. The rats were then gradually food deprived to approximately 80% of their free-feeding weights (375–450 g) and were shaped to lever press for 45 mg food pellets (P. J. Noyes Company, Lancaster, NH) on a continuous reinforcement schedule in a small-animal operant chamber (Model 143-22, BRS/LVE, Beltsville, MD). The number of responses necessary for reinforcement was gradually increased to 20 (FR 20). The operant chamber was enclosed in a custom made environmental isolation chamber which was sound and light attenuating and equipped with a closed-circuit video system (Fig. 1). A computer-based Interact system (BRS/LVE) was programmed to control environmental contingencies and record and reduce the behavioral data. At the termination of each session there was a printout of the number of reinforcers earned and the responses emitted by the rat during each minute. Behavioral sessions were run daily and were approximately one hour in length. When the

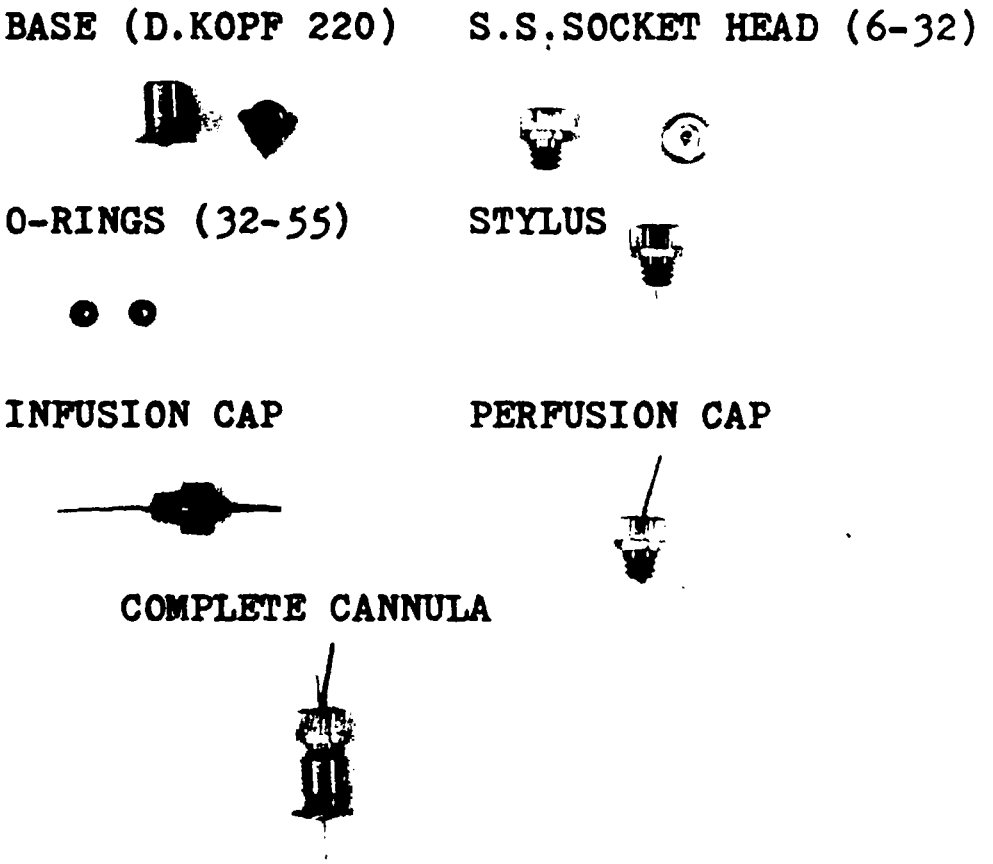

FIG. 2. Parts for and completed push-pull perfusion cannula made of stainless steel and silicone rubber components.

rats' responding was stable they were implanted with perfusion cannulas.

Cannula Construction

Stainless steel injection cannulas (model 220, D. A. Kopf, Tujunga, CA) were modified as follows to serve as perfusion cannula bases (Fig. 2). The needle tubing was removed. The flat portion of the base was trimmed to approximately half its original size. The hole in the bottom of the base was enlarged by drilling to accommodate a length of 20 ga. thin wall stainless steel needle tubing which was secured with polyester retaining compound (No. 35, Loctite, Newington, CT) just inside the well at the bottom of the base. The tubing was then trimmed to extend 6 mm beyond the base. However, the gauge and length of tubing may be varied depending upon the brain perfusion site. Two O-rings (size 32-55, Silicone compound 19711, Precision Associates Incorporated, Minneapolis, MN) were fitted into the base to assure a complete seal between cap and implanted cannula.

All caps were made by securing (with Loctite retaining compound) stainless steel wire or tubing in a hole drilled through a stainless steel socket head screw (1/8th inch, 6/32 thread size). For a stylus, 0.025 inch wire was secured in a screw and trimmed to extend 0.5 to 0.75 mm beyond the tubing in the base when screwed down firmly on the O-rings. A perfusion cap consisted of two pieces of tubing; one length of 30 ga. which extended 0.5 to 0.75 mm through the base (to serve as the push tube), and a shorter piece of 23 ga. tubing which extended 0.5 mm through the cap screw itself (to serve as the pull tube). The 30 ga. push tubing was reinforced by cementing 23 ga. tubing around the 30 ga. tubing above the cap.

The same cannula base can be used as a guide tube in chronic studies to infuse substances into the brain. If it is to be used to simply administer substances directly into the brain, an infusion cap consisting of 23 ga. tubing secured in a screw and trimmed to extend 0.5 to 0.75 mm through the base is sufficient.

Implantation Procedure

A cannula base was permanently implanted in the brain of each rat with the tip in the right lateral ventricle. Animals were anesthetized with sodium pentobarbital (45 mg/kg). Using stereotaxic (Model 900, D. A. Kopf) coordinates for reference [27], the base was positioned perpendicular and anchored to the skull with three stainless steel screws (0-80 thread size, 3/16th inch) and dental acrylic cement. After implantation, the stylus was screwed into the cannula base and the rat was returned to its home cage. The perfusion experiments were begun about 2 weeks after surgery.

Perfusion Procedure

A four channel peristaltic pump (Minipuls II, HP 4, Gilson Electronics, Middleton, WI), used to control the perfusion, was calibrated daily to perfuse at 10 μl/minute. The pump was connected to the perfusion cap with silicone tubing (Dow-Corning Silastic Medical tubing, Dow Corning Corporation, Midland, MI). One piece of tubing, threaded through a 0.060 inch manifold tube (supplied with the pump) in one channel of the pump, connected the push tube to a reservoir containing perfusion medium. Another piece of tubing connected the pull tube, through a manifold tube in a second channel in the pump (in the opposite direction), to a miniature fraction collector. Enough tubing was left between the cannula and the pump to allow the animal free movement in the operant chamber (Fig. 1). The two pieces of tubing between the perfusion cap and the pump were cemented together with silicone adhesive (Dow Corning, Midland, MI) to strengthen the tubing and keep it from becoming tangled.

The first experiment involved perfusion of the rats' lateral ventricles for 40 minutes while they were lever pressing for food reinforcement. Our goal was to determine whether we could recover significant quantities of ^{3}H-DA metabolites in perfusate, upon continuous presentation of the tritiated monoamine. It was deemed necessary to try this *in vivo* superfusion procedure, since preliminary experiments (Sparber, unpublished observations) in which ^{3}H-DA was injected into the ventricle as a bolus were unsuccessful. In those experiments very little unchanged ^{3}H-DA and negligible quantities of metabolites appeared in perfusate starting 5 to 30 minutes after pulse labeling. Our inability to successfully use this method was not surprising in light of a subsequent report that greater than 75% of ^{3}H-DA was no longer present as soon as 5 minutes after injection into the lateral ventricle of the rat [6]. Subsequently, we determined whether d- or l-amphetamine would alter the recovery of metabolites. Amphetamine was chosen because it has a well-defined action on catecholamine neurons (for review see [17]). Four rats were used in this study. The behavior session and perfusion began simultaneously. Approximately 22 minutes later saline was injected (IP). One or two days later d- or l-amphetamine was injected instead of saline. Half of the rats were injected with d-amphetamine first, and then l-amphetamine. The other half of the rats were administered the isomers in the reverse order. At least 6 days separated amphetamine injections.

The second experiment was similar to the first, except there were no injections and the rats' rectal temperature was recorded throughout the session. After cannula base implantation each of the seven rats was adapted to having a temperature probe (Model 402, Yellow Springs Instrument Co., Yellow Springs, OH) inserted approximately 5 cm into its rectum and taped to its tail throughout the behavioral session. Perfusion and behavioral sessions were begun simultaneously and terminated at the end of 40 minutes. Four rats were perfused with the ^{3}H-DA perfusion medium first, followed not less than 4 days later by the ^{3}H-DA perfusion medium plus $PGF_{2\alpha}$ (10 ng/μl). Three rats were perfused with these solutions in the opposite order. Because of the variability between rats in the amount of ^{3}H-DA metabolites in perfusate (*vide infra*), we decided to use each rat as its own control. To determine the effects, if any, of ^{3}H-DA on behavior and rectal temperature, an experiment was performed in which ^{3}H-DA was omitted from the medium.

Changes in behavior [29] or body temperature [24] alter the quantity of catecholamine metabolites in rat brain. $PGF_{2\alpha}$ is not very potent in altering behavior [23] or body temperature [9], but it alters function of catecholamine neurons in the autonomic nervous system [7]. Therefore, we hypothesized that a low concentration of $PGF_{2\alpha}$ would alter the quantity of catecholamine metabolites in perfusate by a direct action on neurons in close proximity to the ventricles as opposed to an indirect action due to effects on behavior or temperature. $PGF_{2\alpha}$ was administered rather than some other PG because it is one of the major PGs in rat brain [1,33]. $PGF_{2\alpha}$ was added to the perfusion medium because systemic injection of PG results in little increase in CNS prostaglandin levels [12,15].

Perfusion Medium

The rats were perfused with a sterile, 0.9% saline solution containing 2.3 mM $CaCl_2$. Tritium labeled DA (^{3}H-2-DA, Spec. Act. 7.5 Ci/mM, New England Nuclear, Boston, MA; 0.1 ng/μl final concentration) or ^{3}H-DA and $PGF_{2\alpha}$ (kindly supplied by Dr. J. Pike, The Upjohn Company, Kalamazoo, MI) were added to the perfusion medium in some experiments. $PGF_{2\alpha}$ was stored in absolute ethanol at −20°C. For administration, an aliquot of the stock solution of $PGF_{2\alpha}$ was evaporated under nitrogen and added to the perfusion medium so that the final concentration of $PGF_{2\alpha}$ was 10 ng/μl.

Collection and Analysis of Perfusate

Sequential 5 minute samples of perfusate were collected into polyethylene microsample tubes, in a small fraction collector, containing 1 μg each of 13 various catecholamines and metabolites in 10 μl 1.0 N formic acid. The following substances were used as cold carriers for subsequent thin-layer chromatographic separation: NA, DA, DOPAC, HVA, 3-MT, MHPG, 3,4-dihydroxyphenylalanine (DOPA), 3-methoxy-4-hydroxyphenylethanolamine (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-hydroxyphenyl-ethanol (HVET). At the conclusion of the behavioral session the animal was disconnected from the perfusion apparatus, the stylus was screwed into the cannula base, and the animal was returned to its home cage.

Perfusate samples 2, 4, 6 and 8 were analyzed using the two-dimensional thin-layer chromatographic separation system of Fleming and Clark [10]. One 10 μl aliquot of each sample was spotted under nitrogen 2.5 cm from the edges, in the lower left corner, of the thin-layer chromatrography plate (20×20 cm glass plates coated with 100 μ microcrystalline cellulose, EM Laboratories, Elmsford, NY). A second 10 μl aliquot was spotted in the upper right corner where it would not be exposed to the solvents (except their vapors). This aliquot was used to control for loss of tritium on drying and visualization. The plates were then developed 13–15 cm in each direction. The separated compounds were visualized with p-nitroaniline [10] and the resulting 14 spots were scraped into counting vials. One-tenth ml of 1.0 N formic acid was added to each vial to elute the tritiated compounds from the cellulose. Ten ml of cocktail (0.9% n-butyl-PBD and 3% BBS-3, Beckman Instruments, Fullerton, CA, in toluene) were added to each vial. Radioactivity (disintegrations per minute, dpm) was determined using liquid scintillation spectrometry and a standard quench curve. Counting efficiency

was approximately 45%. An aliquot of the perfusion medium, collected from the tip of the perfusion cap upon termination of the session, served as a control for background radioactivity present in the perfusion medium. It was added to formic acid containing cold carriers and analyzed as described above.

In order to determine which metabolites were present in significant quantities in perfusate, the amount of radioactivity which cochromatographed with each compound was compared between perfusion medium samples and non-drug, perfusate samples (i.e., sample 4 from the first experiment and samples from perfusion with ^{3}H-DA without $PGF_{2\alpha}$ in the second experiment). Background radioactivity at each spot was consistent across chromatograms from perfusion medium samples when data were expressed as a percentage of the total non-volatile tritium. Because total non-volatile tritium was approximately 10^5 dpm in perfusion medium and perfusate samples alike, the radioactivity found at each compound was calculated as dpm per dpm of non-volatile tritium $\times 10^5$ for each sample. A compound was considered detectable in perfusate if significantly more radioactivity (per dpm non-volatile tritium $\times 10^5$) cochromatographed with it in perfusate samples than in perfusion medium samples.

In the first experiment, ^{3}H-metabolites which were detectable in the perfusate were corrected for the background radioactivity found in the perfusion medium. This net amount of radioactivity for each metabolite from samples (6 and 8) collected after injection of saline or amphetamine was expressed as a percentage of that before injection (in sample 4). Behavioral data were handled similarly, with response rates from periods after saline or amphetamine injection (occurring concurrently with the collection of perfusion samples 6 and 8) expressed as a percentage of that before injection (occurring concurrently with collection of sample 4). Drug effects were determined by comparing results after d- and l-amphetamine with those after saline, using a correlated t-test.

In the second experiment, ^{3}H-metabolites which were detectable in perfusate were corrected for background radioactivity found in the perfusion medium. The average net amount of each ^{3}H-metabolite (in samples 2, 4, 6, and 8), FR 20 behavior and rectal temperature during perfusions with $PGF_{2\alpha}$ in the medium were compared with perfusions without $PGF_{2\alpha}$, using Friedman's one-way analysis of variance by ranks test. Significant differences between treatment means were determined by Wilcoxon's signed rank test.

A critical value of 0.05 was set as that required to indicate a statistically reliable effect of experimental manipulation. Unless otherwise noted, all values are M ± SEM.

RESULTS

FR 20 Behavior

In experiment one, the average rate of lever pressing during the 5 minutes immediately before injection was 1.6 ± 0.1 responses/second. Behavior was unaffected by saline, while injection of either isomer of amphetamine significantly decreased behavior, to a similar extent, within 7.5 minutes after injection and behavior remained suppressed throughout the rest of the session (Table 1).

In experiment two, the average rate of lever pressing for the rats the day before cannula implantation was 1.5 ± 0.2 responses/second. Cannula implantation, the procedure used to measure rectal temperature, or perfusion with the various media (with or without $PGF_{2\alpha}$) had no effect upon behavior.

TABLE 1

d-AND l-AMPHETAMINE DECREASED FIXED-RATIO 20 BEHAVIOR AND THE QUANTITY OF ^{3}H-DOPAC, ^{3}H-HVA AND ^{3}H-MHPG IN RAT BRAIN VENTRICULAR PERFUSATE

	Injection*		
Dependent variable†	Saline	d-Amphetamine (1.5 mg/kg)	l-Amphetamine (3 mg/kg)
Behavior	99 ± 7	1 ± 1‡	10 ± 6‡
^{3}H-DOPAC	192 ± 50	74 ± 24‡	29 ± 14‡
^{3}H-HVA	187 ± 65	112 ± 45‡	47 ± 20‡
^{3}H-MHPG	152 ± 35	68 ± 16‡	62 ± 7‡

*Injections were made (IP) in the midst of the fifth 5 minute segment of the sessions.

†Values are from the eighth 5 minute segment and are expressed as a percentage of the fourth 5 minute segment which occurred immediately prior to the injection. Mean ± SEM (N = 4).

‡$p < 0.05$ compared with saline control sessions; 2-tailed correlated Student's t-test.

Rectal Temperature

In the second experiment the rats' average initial temperature was 37.4 ± 0.2°C and a slight, but significant elevation in temperature (0.6 ± 0.1°) was observed at the end of the 40 minute session. Perfusion with the various media did not prevent or otherwise alter the increase in temperature.

Recovery of Metabolites of ^{3}H-DA and ^{3}H-NA in Perfusate

Perfusion of the lateral ventricular space with a solution containing ^{3}H-DA resulted in recovery of significant quantities of tritium labelled metabolites of ^{3}H-DA and ^{3}H-NA. Tritium which cochromatographed with authentic DOPAC, HVA, 3-MT and MHPG was recovered in quantities significantly above that in perfusion medium not presented to brain (Table 2), while tritium which cochromatographed with HVET, DHPET, DHPG, DHMA, VMA, NA or NM was not significantly elevated. Approximately 85% of the radioactivity recovered from DA and NA metabolites chromatographed with DOPAC and HVA.

There was a great deal of variability between rats in the quantities of metabolites of ^{3}H-DA and ^{3}H-NA in perfusate. However, the ratio of ^{3}H-HVA:^{3}H-DOPAC:^{3}H-3-MT:^{3}H-MHPG was quite similar for all subjects (Table 3).

When either d- or l-amphetamine were administered intraperitoneally, approximately 22 minutes into the perfusion-behavioral session, a concurrent suppression of FR-20 behavior and alteration of ^{3}H-DA metabolites ensued. Figure 3 demonstrates these effects in one rat (M-31). As can be seen, under conditions in which saline was injected (IP) the subject continued to respond at baseline rates. The metabolites of ^{3}H-DA in perfusion samples 6 and 8 were between 100% and 200% of those found in sample 4, which was collected immediately before injection. The effect of the amphetamines upon ^{3}H-3-MT in perfusate was variable and inconsistent, while ^{3}H-DOPAC, ^{3}H-HVA and ^{3}H-MHPG were diminished, especially in sample 8 which was collected approximately 15 minutes after injection (Table 1).

The administration of $PGF_{2\alpha}$ in the perfusion medium decreased the quantities of ^{3}H-DOPAC ($X_R^2 = 13.68$), ^{3}H-HVA ($X_R^2 = 18.90$) and ^{3}H-MHPG ($X_R^2 = 11.80$) recovered from perfusate (Table 2). The effect of $PGF_{2\alpha}$ on ^{3}H-MHPG recovery

TABLE 2

RECOVERY OF SIGNIFICANT QUANTITIES OF METABOLITES OF ^{3}H-DOPAMINE AND ^{3}H-NORADRENALINE IN RAT BRAIN PERFUSATE. $PGF_{2\alpha}$ DECREASED THE QUANTITY OF ^{3}H-DOPAC, ^{3}H-HVA, AND ^{3}H-MHPG IN PERFUSATE

	^{3}H-DA perfusion medium	^{3}H-DA perfusate	^{3}H-DA + $PGF_{2\alpha}$ perfusate
^{3}H-HVA	7 ± 2*	495 ± 174†	88 ± 40‡
^{3}H-DOPAC	17 ± 5	331 ± 111†	43 ± 12‡
^{3}H-3-MT	21 ± 2	107 ± 26†	50 ± 13
^{3}H-MHPG	18 ± 2	41 ± 10†	26 ± 1

*Quantities of ^{3}H-metabolites are expressed as dpm per dpm of nonvolatile tritium × 10^5. Nonvolatile tritium averaged 1.2 (±0.1) × 10^5 dpm (mean ± SD, n−14) in perfusion medium, 1.1 (±0.1) × 10^5 dpm (n=7) in ^{3}H-DA perfusate and 1.1 (±0.1) × 10^5 dpm (n=7) in ^{3}H-DA perfusate with $PGF_{2\alpha}$. Perfusate data are the average of values from perfusion samples 2, 4, 6, and 8. Mean ± SEM.

†$p<0.05$ compared with samples of the perfusion medium that were not exposed to the rats' brains, but otherwise were analyzed in a manner identical to the perfusate. (Friedman's one-way analysis of variance by ranks test and Wilcoxon's signed rank test.)

‡$p<0.05$ compared with the appropriate data from experiments where the perfusion medium contained only ^{3}H-DA (Friedman's one-way analysis of variance by ranks test and Wilcoxon's signed ranks test).

TABLE 3

VARIABILITY AMONGST RATS IN THE QUANTITY, BUT CONSISTENCY IN THE RATIO, OF METABOLITES OF ^{3}H-DOPAMINE IN RAT BRAIN VENTRICULAR PERFUSATE

Rat	^{3}H-HVA	^{3}H-DOPAC	^{3}H-3-MT	^{3}H-MHPG	Ratio
1	83*	50	25	10	3 : 2 : 1 : 0.4
2	1251	737	128	92	10 : 6 : 1 : 0.7
3	1004	673	280	140	4 : 2 : 1 : 0.5
4	91	54	25	14	4 : 2 : 1 : 0.6
5	195	135	48	4	4 : 3 : 1 : 0.1
6	608	487	102	23	6 : 5 : 1 : 0.2
7	241	170	50	4	5 : 3 : 1 : 0.1

*Quantities of ^{3}H-metabolites are expressed as dpm per dpm non-volatile tritium × 10^5. Non-volatile tritium averaged 1.1 (±0.1) × 10^5 dpm (mean ± SD, n−7). Values represent the average of data from perfusion samples 2, 4, 6 and 8, and have been corrected for blank values.

is evident from the finding that inclusion of $PGF_{2\alpha}$ in the perfusion medium prevented the recovery of significant quantities of ^{3}H-MHPG. To ensure that these effects of $PGF_{2\alpha}$ were not due to its interfering with the assay for ^{3}H-DOPAC, ^{3}H-HVA or ^{3}H-MHPG, perfusion samples were divided, $PGF_{2\alpha}$ (500 ng) was added to half of each sample, and all samples were assayed as described previously. This *in vitro* addition of $PGF_{2\alpha}$ had no effect on the recovery of any of the ^{3}H-metabolites.

DISCUSSION

Perfusion of the lateral ventricular space with a solution containing ^{3}H-DA resulted in recovery of radioactivity which cochromatographed with authentic DOPAC, HVA, 3-MT, and MHPG. None of the other DA or NA metabolites analyzed appeared in perfusate in quantities significantly above blank levels. The major portion of the metabolites recovered were ^{3}H-DOPAC and ^{3}H-HVA. These findings are in agreement with reports that the major metabolites of DA in the CNS are the acid metabolites DOPAC and HVA, while the major metabolite of NA in the CNS is MHPG (for review see Sharman [26]). The fact that we did not recover significant quantities of the minor metabolites of ^{3}H-DA and ^{3}H-NA suggests that we were not measuring some nonspecific metabolism of ^{3}H-DA, but were probably observing reflections of changes in dopaminergic and noradrenergic neuronal function.

There was a great deal of variability between rats in the amount of metabolites of ^{3}H-DA in perfusate. This variability may be due to one or more of a number of factors. Small differences in the placement of the cannula in the ventricle may change the amount of ^{3}H-DA taken into neurons and therefore the amount of ^{3}H-DA available for metabolism intraneuronally. Even if the same amount of ^{3}H-DA were taken into neurons in all rats, there are likely to be individual differences in dopamine turnover rates. To control for this individual variability, we used a within-subjects experimental design, each animal serving as its own control.

Braestrup and coworkers [6] found that intraventricular injection of ^{3}H-DA resulted in the formation of ^{3}H-HVA, ^{3}H-DOPAC, ^{3}H-3-MT and ^{3}H-MHPG, in the ratio of about 70:70:10:1, in rat brain tissue. We found the ratio of these compounds in rat brain ventricular perfusate was similar, about 50:30:10:2. This suggests that some of the ^{3}H-DA pushed into the ventricles was reaching brain tissue and being metabolized in a manner similar to ^{3}H-DA injected into the ventricles. Additionally, some of the ^{3}H-DA was metabolized to ^{3}H-NA and subsequently to ^{3}H-MHPG.

Myers and Mora [20] prelabeled the medial prefrontal cortex of unanesthetized rats with 1.65 μg ^{14}C-DA and then perfused the site with artificial cerebrospinal fluid. Thirty minutes after the beginning of the perfusion, a time corresponding to the collection of perfusate in the experiments described above, they found ^{14}C-DOPAC, ^{14}C-HVA and ^{14}C-3-MT in perfusate in a ratio of about 40:20:3. They did not assay for ^{14}C-MHPG. Their data were quite similar to ours, and supports the suggestion that DOPAC and HVA are the major metabolites of DA in unanesthetized rats.

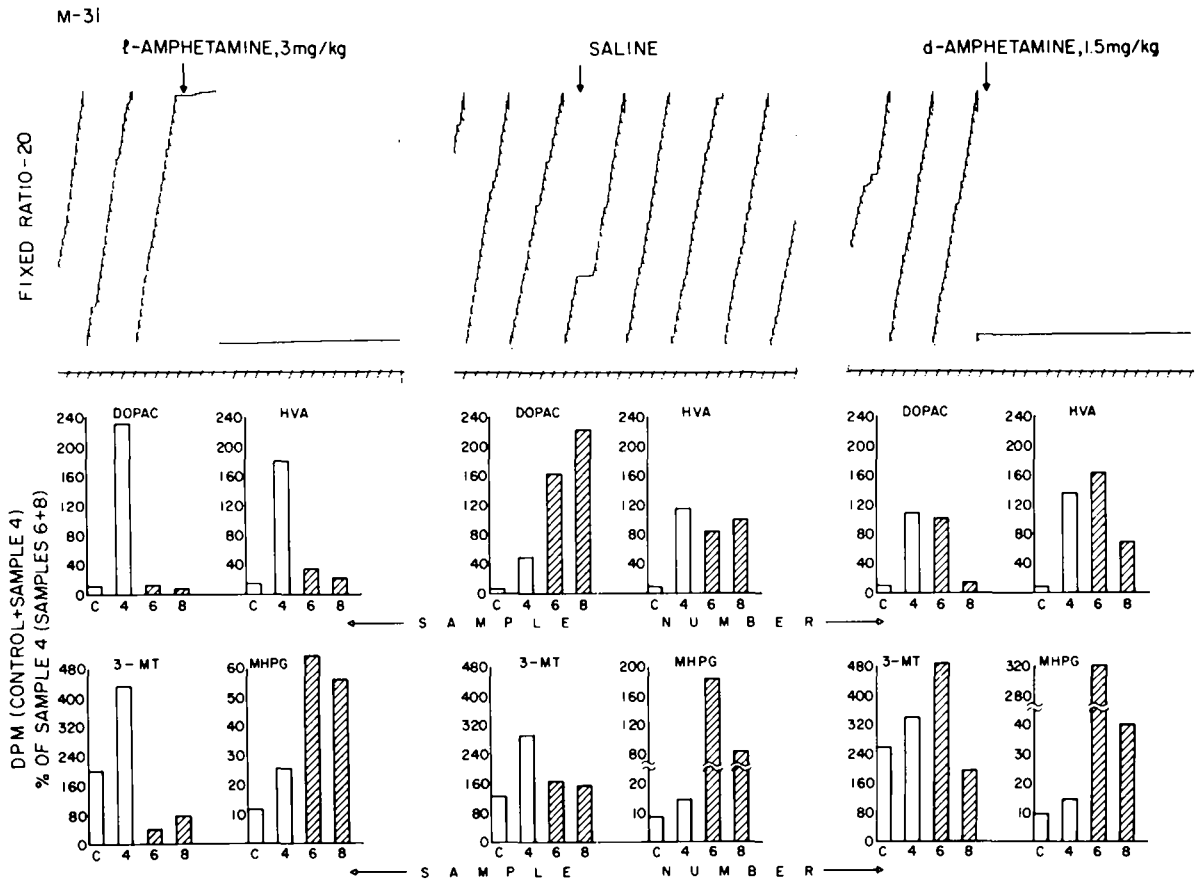

FIG. 3. Concurrent effects of d- and l-amphetamine on fixed ratio 20 behavior and recovery of ^{3}H-dopamine metabolites in perfusate from rat M-31. Rat M-31 was perfused with a solution containing ^{3}H-dopamine for 40 minutes. At the same time its behavior was controlled by a fixed-ratio 20 schedule of food reinforcement. Amphetamine or saline was injected 22 minutes into the sessions: a time corresponding to the collection of the fifth perfusion sample and indicated on the cumulative records by arrows. The hatch marks at the bottom of the behavioral records indicate intervals of one minute. Sample numbers represent sequential 5 minute samples. Sample C represents background radioactivity found in the perfusion medium (not exposed to brain) for the metabolites depicted. Sample 4 (open bars) represent the quantity of recovered tritiated compound in the perfusate just prior to injection. The quantity of ^{3}H is expressed as dpm per dpm non-volatile tritium$\times 10^5$. Non-volatile tritium averaged 1.3 ($\pm$0.1)$\times 10^5$ dpm (M $\pm$ SD, n=3) for perfusion medium (sample C) and 1.2 ($\pm$0.1)$\times 10^5$ dpm (M + SD, n=9) for perfusate samples (4, 6 and 8). The striped bars, samples 6 and 8, represent the amount of metabolite in the perfusate as a percentage of sample 4, the values on the ordinates being the same for both dpm and %.

The first experiment resulted in evidence which indicated that ^{3}H-DA metabolites could be systematically altered as a consequence of amphetamine injection. It did not allow us to conclude that the cortical stimulant was directly affecting the disposition or metabolism of transmitter(s) or if the change in metabolic profile was secondary to behavioral changes, which in turn may have been responsible for changes in catecholaminergic activity [25,28]. However, it was not our intention to determine whether amphetamine alters catecholaminergic activity; countless *in vitro* and *in vivo* demonstrations of this effect have already been published. We used the amphetamines as a positive control to validate the continuous perfusion procedure. Amphetamine should lower the levels of acid metabolites of ^{3}H-DA by facilitating release and secondarily blocking reuptake (uptake) of ^{3}H-DA, thereby diminishing its accessability to intraneuronal monoamine oxidase. We had to verify that extracellular ^{3}H-DOPAC and ^{3}H-HVA (i.e. that found in perfusate) would reflect this situation, especially in a dynamic system. Because the actions of PG's in the CNS are little understood, unlike those of amphetamine, we chose to control for potential indirect effects of behavior and/or body temperature upon ^{3}H-DA metabolism by using a behaviorally and thermically inactive dose of $PGF_{2\alpha}$.

$PGF_{2\alpha}$ decreased the quantities of ^{3}H-DOPAC, ^{3}H-HVA and ^{3}H-MHPG in perfusate without changing the rats' fixed-ratio behavior or rectal temperature. More recently we have observed that higher doses of $PGF_{2\alpha}$ significantly decreased the quantities of ^{3}H-DOPAC, ^{3}H-HVA, ^{3}H-3-MT and ^{3}H-MHPG in perfusate without affecting fixed-interval behavior or rectal temperature (Nielsen and Sparber, in preparation). This suggests that $PGF_{2\alpha}$ is affecting CNS dopaminergic and noradrenergic function without having to alter physiological or behavioral variables, thereby ruling out, to a great extent, the possibility that changes in the metabolic profile were indirectly induced. $PGF_{2\alpha}$'s actions on CA neurons can be

interpreted by comparison with amphetamine's effects. Amphetamine is known to increase the release and decrease the reuptake of DA and NA (for review see Lewander [17]). In our hands, d- and l-amphetamine decreased the amounts of radiolabelled DOPAC, HVA and MHPG in ventricular perfusate. This would suggest that compounds like $PGF_{2\alpha}$ which produce this metabolic profile are also altering the release and/or reuptake of catecholamines.

The push-pull perfusion technique has been criticized because of the tissue damage produced by cannula implantation and perfusion [5,16]. Although the existence of some damage to cerebral tissue cannot be denied, we found that neither the implantation procedure nor perfusion with or without ^{3}H-DA (1 ng/10 μl/minute) affected behavior or temperature. However, at this rate of perfusion and concentration of ^{3}H-DA, we found quantities of ^{3}H-DA and ^{3}H-NA metabolites significantly above blank and in the same relative amounts reported in brain tissue using other methodologies. Additionally, we were able to recover metabolites with or without concurrent changes in FR 20 behavior, thereby indicating that changes in behavior and neurochemistry can occur and be monitored together, but that they do not necessarily covary. There are certainly limitations in determining the mechanism of drug effect based on changes in the amount of metabolites of putative neurotransmitters in perfusate. Our approach to this problem involves comparing the effects of unknown drugs, eg. PGs, with drugs having well-known mechanisms of action, eg. amphetamine [31]. Nevertheless, within the limits of the methodology, we feel that push-pull perfusion of the lateral ventricles is a useful technique for monitoring ongoing neurochemical and behavioral events concurrently.

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