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EFFECTS OF AMPHETAMINE ON THE CONTENTS OF NOREPINEPHRINE AND ITS METABOLITES IN THE EFFLUENT OF PERFUSED CEREBRAL VENTRICLES OF THE CAT*

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Abstract—One hr after the injection of *dl*-^{3}H-norepinephrine into various regions of the cat cerebroventricular system, the ventricles were perfused with an artificial cerebrospinal fluid (CSF). This perfusing fluid was pumped into the left lateral ventricle at a rate of 0·1 ml/min and collected at 10-min intervals from a catheter in the cerebral aqueduct or in the cisterna magna. Two hr after the perfusion was initiated the amount of radioactivity in the effluent remained relatively steady. At this time the ventricular system was perfused for 30 min with CSF containing various concentrations of *d*- or *l*-amphetamine. *d*-Amphetamine sulfate (25–400 μg/ml) caused an immediate increase in the content of ^{3}H-norepinephrine in the effluent; after a latent period of 10–20 min there was also a significant increase in the effluent content of ^{3}H-normetanephrine while the concentration of deaminated-*O*-methyl metabolites did not change. *l*-Amphetamine sulfate (50 μg/ml) did not significantly increase the amount of ^{3}H-norepinephrine or ^{3}H-normetanephrine in the perfusion effluent. *d*-Amphetamine caused a greater increase in the effluent concentration of ^{3}H-norepinephrine and ^{3}H-normetanephrine when the amine was injected into the lateral ventricle than when it was injected into the third ventricle or cisterna magna. Intravenous injections of *d*-amphetamine (1 mg/kg) also increased the content of ^{3}H-norepinephrine in the perfusion effluent. After injections of ^{3}H-dopamine into the lateral ventricle, *d*-amphetamine increased the efflux of both ^{3}H-norepinephrine and ^{3}H-dopamine, whereas after the intraventricular administration of ^{14}C-inulin or ^{14}C-urea, the effluent content of these inert substances was not increased by *d*-amphetamine. These results indicate that amphetamine increases the efflux of catecholamines from structures bordering the cerebroventricular system by blocking the reuptake process, or enhancing the release mechanism, or both.

BEFORE norepinephrine can be considered a neurotran mitter in the central nervous system it should be demonstrated that this amine is released from noradrenergic neurons in the brain or spinal cord in response to nerve impulses.[1] Although several investigators have demonstrated the release of ^{3}H-norepinephrine from brain preparations *in vitro* after the addition of drugs,[2, 3] or the application of electrical pulses,[4, 5] there have been relatively few studies dealing with the release of norepinephrine from brain *in situ*.

Several investigators have demonstrated the release of norepinephrine or dopamine

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from the brain using a push–pull cannula.[6–8] With this technique, Chase and Kopin[9] recently demonstrated that odor stimuli released norepinephrine from the olfactory bulb of rats. However, these stimuli also caused the release of inert substances (inulin, urea) which are unlikely neurotransmitters. The authors suggested that their results were artifacts of the push–pull cannulation technique. Indeed, this technique has the disadvantage that the area being perfused is damaged and thereby forms an artificial extracellular space.[9, 10] To circumvent this problem a cerebroventricular perfusion technique, similar to that reported by Carmichael *et al.*,[11] was used in the present study in order to detect the release of ^{3}H-norepinephrine and its metabolites from various brain regions.

Since amphetamine releases norepinephrine from peripheral sympathetic nerve endings[12] and lowers the brain content of norepinephrine,[13, 14] it has been suggested that this drug exerts its behavioral stimulation by releasing catecholamines from nerve terminals in the brain.[15] Indeed, using the push–pull cannulation technique, it has been demonstrated that amphetamine releases dopamine[16] and norepinephrine[8] from the brain. In the present report, by utilizing a cerebral ventricular perfusion technique in conjunction with the intraventricular injection of ^{3}H-norepinephrine, we have monitored the release of norepinephrine from the brain *in situ* and examined the effects of amphetamine upon this release. Preliminary studies of this work have been reported previously.[17, 18]

METHODS

Mongrel cats of either sex weighing 2–4 kg were briefly anesthetized by open-drop administration of methoxyflurane and placed in a stereotaxic apparatus (David Kopf, Inc.) where they remained for the entire experimental period. The spinal cord was sectioned at C_1 and respiration was maintained with a respirator pump. Systemic blood pressure was recorded from the right carotid artery and rectal temperature was maintained at 37·5° with a heating pad.

A self-tapping screw-type cannula, with a dead space of 6 to 8 μl, was implanted into either the anterior horn of the left lateral cerebral ventricle or the ventral portion of the third ventricle as described by McCarthy and Borison.[19] Five μc of *dl*-norepinephrine-7-^{3}H hydrochloride (9·71 c/mM, 1 μc/μl) or 15 μc of ^{3}H-dopamine (generally labeled; 5 c/mM, 1 μc/μl) obtained from New England Nuclear Corp. was injected intraventricularly. These solutions were immediately flushed out of the cannula with 10 μl of an artificial cerebrospinal fluid[20] so that the labeled amines were given in an effective volume of approximately 10 or 20 μl. In four experiments, 0·1 μc of inulin-carboxyl-^{14}C (0·005 μc/μl) or 1·0 μc of urea-^{14}C (0·27 mc/mM, 0·05 μc/μl) were injected into the lateral ventricle and flushed out of the cannula with 10 μl of artificial cerebrospinal fluid. One hr after the injection of ^{3}H-norepinephrine and 2 hr after the injection of ^{3}H-dopamine the cisterna magna was surgically exposed and a polyethylene cannula (4 cm × 2 mm, o.d.) was passed along the floor of the fourth ventricle and into the cerebral aqueduct. Artificial cerebrospinal fluid was then infused into the lateral ventricle at a rate of 100 μl per min with a Harvard infusion pump. The effluent was collected from the aqueduct cannula in 5-ml glass-stoppered centrifuge tubes containing 0·1 ml of 5·0 N acetic acid for successive 10-min periods. In 3 experiments where ^{3}H-norepinephrine was injected into the cisterna, the perfusion effluent was collected from a 20-gauge needle inserted into the cisterna magna of

cats which were anesthetized with an intraperitoneal injection of a urethane–barbiturate mixture (Dial-Urethane, Ciba); sodium diallyl-barbiturate (70 mg/kg), urethane (280 mg/kg) and monoethylurea (280 mg/kg).

Various amounts of *d*- and *l*-amphetamine sulfate were dissolved in artificial cerebrospinal fluid and perfused through the cerebroventricular system for 30-min periods. In three experiments, *d*-amphetamine SO_4 was dissolved in saline and given intravenously (1 mg/kg).

After the perfusion period, 5 μl of 1 % methylene blue was injected intraventricularly to confirm the cannula tip position. The chest was opened along the midline and entire vascular system was perfused with saline by means of a catheter placed into the aorta through an incision in the left ventricle. Blood was washed out through the incised right atrium. The brain was then quickly removed from the skull and the left caudate nucleus, hypothalamus and septal area were dissected and weighed. In the experiments involving cisternal injections, the brain stem was also taken.

The tissues were homogenized in 4 ml (12 ml for brain stem) of cold 0·4 N perchloric acid and analyzed for their contents of ^{3}H-norepinephrine, ^{3}H-normetanephrine, ^{3}H-deaminated catechols and ^{3}H-deaminated-*O*-methyl metabolites, as described previously.[21]

The effluent samples were analyzed for ^{3}H-norepinephrine, ^{3}H-dopamine and their metabolites in the following manner. One hundred mg of washed aluminium oxide (Woelm) and 0·1 ml of 0·2 M ethylenediamine tetracetate were added to each sample tube. After adjusting the pH to 8·6 with 0·5 and 0·2 N potassium hydroxide, the tubes were shaken and then centrifuged. The supernatant (alumina effluent) was set aside and subsequently assayed for *O*-methylated metabolites (see below). The alumina was then washed with 1·0 ml of 0·2 M sodium acetate and with 1 ml of distilled water. Labeled catechols were eluted with 1 ml of 0·5 N acetic acid.

Five min of shaking were used in all wash steps and 10 min of shaking in the elution step. Thin-layer chromatographic analysis[21] revealed that deaminated catechols always represented less than 10 per cent of the total radioactivity in the effluent sample; accordingly, after ^{3}H-norpinephrine injections, all radioactivity in the alumina eluate was expressed as ^{3}H-norepinephrine (alumina recovery was 54·5 $\pm$ 1·9 per cent). In those experiments in which ^{3}H-dopamine was injected, the catecholamines in the alumina eluate were separated by thin-layer chromatograhy[21] (recovery of norepinephrine was 25·8 $\pm$ 2·8 per cent and of dopamine was 37·9 $\pm$ 3·1 per cent. One hundred μl of the alumina eluate or 4 cm^2 of the thin-layer sheet containing the appropriate spot was added to scintillation vials containing 1 ml of water and 10 ml of modified Bray's solution (6 g of 2, 5-diphenyloxazole and 100 g of naphthalene per liter of dioxane) and counted in a Beckman DPM-100 liquid scintillation spectrometer. ^{3}H-norepinephrine and ^{3}H-dopamine were corrected for counting efficiency (30 per cent) and chromatographic recovery.

The effluent from the alumina was adjusted to pH 6 with 0·2 N acetic acid and placed on columns of Dowex 50W $\times$ 8 (H^+ form, 6 $\times$ 40 mm). The columns were washed with 5 ml of distilled water and this wash was added to the Dowex effluent. The radioactivity in 100 μl of the combined effluent-wash represented the deaminated-*O*-methyl metabolites (counting efficiency was 30 per cent). ^{3}H-nor-metanephrine was eluted from the column with 5 ml of a 1:1 mixture of 6 N HCl and ethanol. Radioactivity in the eluate (100 μl) was corrected for counting efficiency (13 per cent)

and chromatographic recovery of a ^{3}H-normetanephrine standard (70·9 ± 2·1 per cent).

Statistical analysis of the concentration of labeled compounds in perfusion effluents was carried out using Student's *t*-test, paired comparison.[22]

RESULTS

Perfusion of cerebral ventricles with artificial cerebrospinal fluid after intraventricular injection of ^{3}H-norepinephrine. The washout pattern of ^{3}H-norepinephrine and its metabolites after the injection of this amine into the left lateral cerebral ventricle is shown in Fig. 1. In these control (no drug) experiments, artificial cerbrospinal-fluid was perfused through the left lateral and third ventricles 1 hr after ^{3}H-norepine

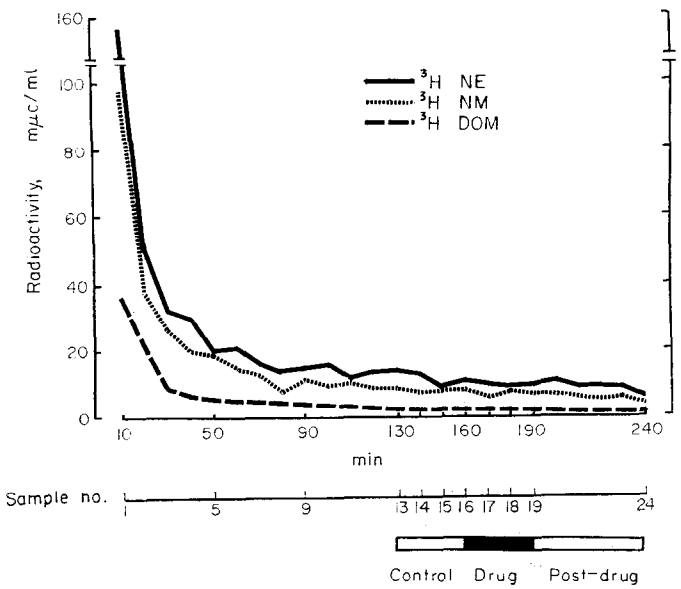

FIG. 1. Time course of the concentration of ^{3}H-norepinephrine (^{3}H NE), ^{3}H-normetanephrine (^{3}H NM), and ^{3}H-deaminated-*O*-methyl metabolites (^{3}H DOM) in the perfusion effluent during 2 control experiments. Five μc of ^{3}H-norepinephrine was injected into the left lateral cerebral ventricle of a spinal-sectioned cat. Perfusion of the left and third cerebral ventricles was begun 1 hr later. After 4 hr of perfusion, the effluent samples were assayed as described in Methods. The experimental procedure employed in all subsequent experiments is shown at the bottom of the figure. After 2 hr of perfusion, three control effluent samples were collected (sample nos. 14–16) and a drug solution was given by intraventricular perfusion for the next 30 min (sample nos. 17–19). This was followed by the collection of 5 postdrug samples (sample nos. 20–24).

phrine had been injected. Within 2 hr the concentration of ^{3}H-norepinephrine ^{3}H-normetanephrine and ^{3}H-deaminated-*O*-methyl metabolites in the effluent had reached a relatively steady concentration. The experimental procedure outlined at the bottom of Fig. 1 was employed in all of the subsequent experiments in which amphetamine was given.

Effect of d-amphetamine on the concentration of ^{3}H-norepinephrine and its metabolites in the perfusion effluent. The results of a typical experiment on the time course of the effects of *d*-amphetamine on the effluent content of ^{3}H-norepinephrine and its metabolites is compared with a control (no drug) experiment in Fig. 2. The lateral and third ventricles were perfused with artificial cerebrospinal fluid alone or with *d*-amphetamine SO_4 (100 μg/ml; total dose 300 μg) during the time period indicated DRUG in Fig. 1. Amphetamine caused an immediate increase in the concentration of ^{3}H-norepinephrine; 30 min after discontinuing the *d*-amphetamine perfusion the concentration of ^{3}H-norepinephrine in the effluent was still elevated above predrug

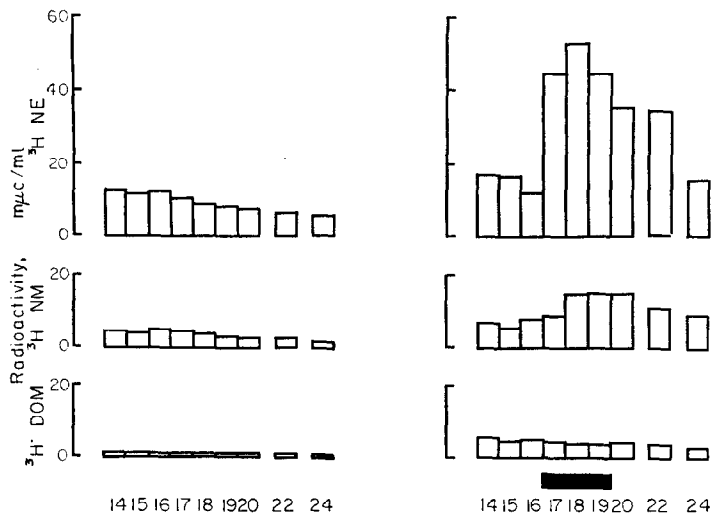

FIG. 2. Effects of *d*-amphetamine SO_4 on the concentration of ^{3}H-norepinephrine and its metabolites in cerebroventricular effluent. The graphs on the left represent concentrations of ^{3}H-norepinephrine (^{3}H NE), ^{3}H-normetanephrine (^{3}H NM) and ^{3}H-deaminated-*O*-methyl metabolites (^{3}H DOM) in the perfusion effluent during a typical control perfusion. Those on the right represent concentrations during an experiment in which *d*-amphetamine SO_4 (100 μg/ml; total dose, 300 μg) was perfused during the time period indicated by the solid horizontal bar below the graph. The height of each bar represents the concentration in effluent collected during a 10-min period.

values. *d*-Amphetamine also increased the concentration of ^{3}H-normetanephrine appearing in the effluent after a latent period of 10–20 min, while the level of ^{3}H deaminated-*O*-methyl metabolites remained unchanged throughout the perfusion period. Figure 3 illustrates the relationship between the dose of *d*-amphetamine SO_4 and the increase in the amount of ^{3}H-norepinephrine and ^{3}H-normetanephrine in the perfusion effluent. Because of variability in the results of the small number of experiments, a smooth dose-response curve was not obtained. However, perfusion with 25 μg/ml of *d*-amphetamine SO_4 significantly increased the amount of ^{3}H-norepinephrine appearing in the effluent. Although the effects of 50 μg/ml of *d*-amphetamine SO_4 were significantly greater than those obtained with 25 μg/ml, there were no significant differences among the increased ^{3}H-norepinephrine values obtained with 50–400 μg/ml of *d*-amphetamine SO_4. Similar but less pronounced

effects were seen with ^{3}H-normetanephrine. Because of the delay in the amphetamine-stimulated increase in the effluent concentration of ^{3}H-normetanephrine, sample nos. 18, 19 and 20 rather than 17, 18 and 19 compared with the predrug samples. When compared in this manner, a significant increase in the amount of ^{3}H-normetanephrine appearing in the effluent was obtained when 50 μg/ml or more of *d*-amphetamine SO_4 was perfused.

When the content of ^{3}H-norepinephrine and its metabolites were measured in the caudate nucleus, hypothalamus and septal area the concentrations after perfusion

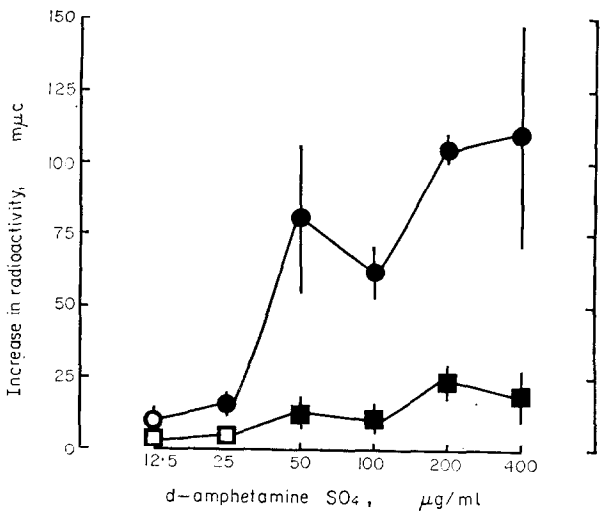

FIG. 3. Dose-response curve for the effects of *d*-amphetamine SO_4 on the concentration of ^{3}H-norepinephrine and ^{3}H-normetanephrine in the perfusion effluent. Each point represents the mean increase (vertical lines denote 1 standard error) in ^{3}H-norepinephrine (O) in sample nos. 17–19 and in ^{3}H-normetanephrine (□) in sample nos. 18–20 over the three control samples (sample nos. 14–16) as determined from three to five animals. Where no vertical line is shown, the standard error is less than the radius of the point. Solid figures represent those values that are significantly different from the predrug values ($P < 0{\cdot}05$).

of *d*-amphetamine (25–400 μg/ml) were not significantly different from those after the control (no drug) perfusions. The failure of amphetamine to reduce the concentration of ^{3}H-norepinephrine was probably because of the variation in the initial tissue concentrations of this amine.

Effect of l-amphetamine on the concentration of ^{3}H-norepinephrine and ^{3}H-normetanephrine in the perfusion effluent. A complete dose–response curve for *l*-amphetamine was not obtained, but in Table 1 the effects of 50 μg per ml of *d*- and *l*-amphetamine SO_4 on the concentration of ^{3}H-norepinephrine and ^{3}H-normetanephrine in the perfusion effluent are compared. At this dose a significant increase in the concentration of ^{3}H-norepinephrine was observed during the perfusion of the *d*- but not with the *l*-isomer of amphetamine. When sample nos. 18, 19 and 20 were compared with 14, 15 and 16, there was a significant increase in the concentration of ^{3}H-normetanephrine only after administration of *d*-amphetamine.

TABLE 1. EFFECTS OF *d*- AND *l*-AMPHETAMINE SO_4 ON THE CONCENTRATION OF ^{3}H-NOREPINEPHRINE AND ^{3}H-NORMETANEPHRINE IN THE PERFUSION EFFLUENT*

Drug	N†	^{3}H-norepinephrine (mμc ± S.E.)	^{3}H-normetanephrine (mμc ± S.E.)
d-Amphetamine	4	80·7 ± 25·4‡	7·5 ± 4·6‡
l-Amphetamine	4	−4·6 ± 20·1	1·0 ± 6·5

*One hr after the injection of ^{3}H-norepinephrine into the left lateral ventricle, *d*-amphetamine (50 μg/ml) or *l*-amphetamine (50 μg/ml) was perfused through the left lateral and third ventricles according to the procedure described in Fig. 1. Each value represents the mean increase in radioactivity for ^{3}H-norepinephrine (sample nos. 17–19) and ^{3}H-normetanephrine (sample nos. 18–20) over the three predrug samples (14–16).

†N = Number of animals.

‡Mean for drug samples significantly greater than predrug samples ($P < 0.05$).

TABLE 2. EFFECT OF INJECTION SITE ON THE INCREASE IN ^{3}H-NOREPINEPHRINE AND ^{3}H-NORMETANEPHRINE IN THE PERFUSION EFFLUENT INDUCED BY *d*-AMPHETAMINE*

Injection site	N†	^{3}H-norepinephrine (mμc ± S.E.)	^{3}H-normetanephrine (mμc ± S.E.)
Left lateral ventricle	4	80·7 ± 25·4	7·5 ± 4·6
Third ventricle	3	18·9 ± 9·9	0·5 ± 0·5
Cisterna	3	1·8 ± 1·0	0·1 ± 0·1

*One hr after the injection of 5 μc of ^{3}H-norepinephrine into the left lateral ventricle, third ventricle, or cisterna magna, the ventricular system was perfused with artificial cerebrospinal fluid and *d*-amphetamine SO_4 (50 μg/ml) as described in Fig. 1. Each value represents the mean increase in radioactivity for ^{3}H-norepinephrine (sample nos. 17–19) and ^{3}H-normetanephrine (sample nos. 18–20) over the 3 predrug samples (14–16).

†N = Number of animals.

Effect of injection site on the release of ^{3}H-norepinephrine and ^{3}H-normetanephrine by d-*amphetamine.* To determine the primary sites of amphetamine-induced release of labeled compounds, ^{3}H-norepinephrine was injected into various regions of the cerebroventricular system: left lateral ventricle, ventral portion of the third ventricle, cisterna magna. One hr after each injection, artificial cerebrospinal fluid and *d*-amphetamine SO_4 (50 μg/ml) were perfused through the ventricles according to the procedure described in Fig. 1 and in Methods. The increase in ^{3}H-norepinephrine and ^{3}H-normetanephrine in the perfusion effluents is shown in Table 2. A significantly greater amount of both amines was detected in the effluent after lateral ventricle injections into the other sites. The contents of ^{3}H-norepinephrine in the left caudate nucleus, hypothalamus, septal area, and brain stem after the perfusion experiments are summarized in Table 3. After cisternal injections, significant quantities of ^{3}H-norepinephrine were found only in the brain stem. The concentration of ^{3}H-norepinephrine was higher in the hypothalamus and septal area after the amine was injected into the third ventricle than when it was injected into the lateral ventricle (see also reference 21). Nevertheless, more ^{3}H-norepinephrine appeared in the effluent after lateral than after third ventricular injections of the labeled compound. Thus, much of the ^{3}H-norepinephrine that is detected in the effluent after perfusion with *d*-amphetamine must originate from structures lining the lateral ventricle.

TABLE 3. ^{3}H-NOREPINEPHRINE CONTENT OF VARIOUS BRAIN TISSUES AFTER INJECTION INTO DIFFERENT CEREBROVENTRICULAR SITES*

Injection site	N†	L. caudate (mμc/g ± S.E.)	Hypothalmus (mμc/g ± S.E.)	Septal area (mμc/g ± S.E.)	Brain stem (mμc/g ± S.E.)
Left lateral ventricle	4	1565 ± 329	232 ± 44	267 ± 45	‡
Third ventricle	3	96 ± 55	952 ± 229	1036 ± 531	‡
Cisterna	3	‡	‡	‡	46 ± 25

* One hr after the injection of 5 μc of ^{3}H-norepinephrine into the left ventricle, third ventricle, or cisterna magna, the cerebroventricular system was perfused with artificial cerebrospinal fluid and *d*-amphetamine SO_4 (50 μg/ml) as described in Fig. 1. The animals were then sacrificed and various brain tissues were analyzed for ^{3}H-norepinephrine.
†N = Number of animals.
‡Radioactivity was not significantly greater than background.

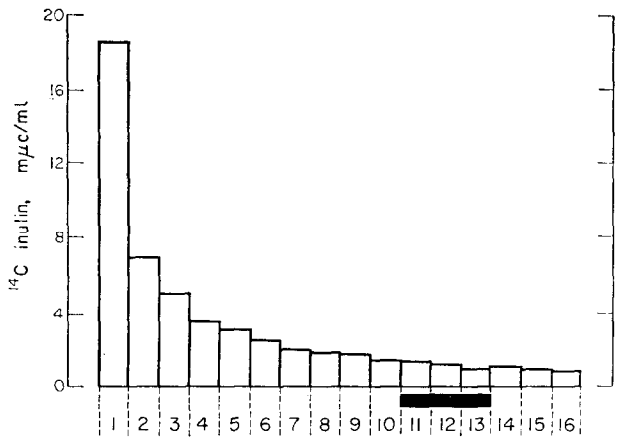

FIG. 4. Effect of *d*-amphetamine SO_4 on the concentration of ^{14}C-inulin in the perfusion effluent. The height of each bar represents the average concentration of ^{14}C-inulin in effluent collected over a 10-min period from two experiments. *d*-Amphetamine SO_4 (50 μg/ml) was perfused during the time period indicated by the solid horizontal bar below the graph.

Effect of d-*amphetamine on the content of* ^{14}*C-inulin and* ^{14}*C-urea in the perfusion effluent.* To determine the specificity of the amphetamine-induced release of ^{3}H-norepinephrine, 0·1 μc of ^{14}C-inulin or 1 μc of ^{14}C-urea was injected into the left lateral ventricle; perfusion of the ventricular system was begun 1 hr later. After 100 min of control perfusion *d*-amphetamine SO_4 (50 μg/ml) was added to the CSF and this solution was perfused for 30 min. Amphetamine had no significant effect on the efflux of either ^{14}C-inulin (Fig. 4) or ^{14}C-urea (Fig. 5).

Effect of d-*amphetamine on the concentration of* ^{3}*H-norepinephrine,* ^{3}*H-dopamine and* ^{3}*H-O-methyl amines in the perfusion effluent after the intraventricular injection of* ^{3}*H-dopamine.* Figure 6 shows the effects of a 30-min perfusion of *d*-amphetamine (50 μg/ml) on the efflux of labeled catecholamines and their metabolites after injection of 15 μc of ^{3}H-dopamine into the left ventricle. *d*-Amphetamine significantly increased the concentration of both ^{3}H-dopamine and ^{3}H-norepinephrine; the latter amine must have been synthesized *in vivo* from the ^{3}H-dopamine. As in the ^{3}H-norepinephrine

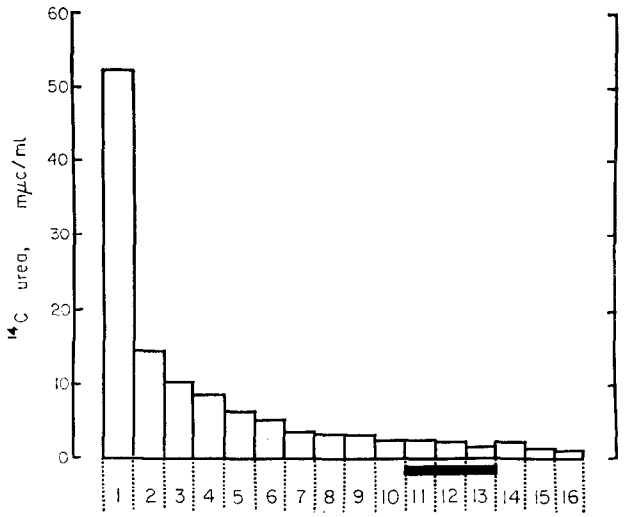

FIG. 5. Effect of *d*-amphetamine SO_4 on the concentrations of ^{14}C-urea in the perfusion effluent. The height of each bar represents the average concentration of ^{14}C-urea in effluent collected over a 10-min period from two experiments. *d*-Amphetamine SO_4 (50 μg/ml) was perfused during the time period indicated by the solid horizontal bar below the graph.

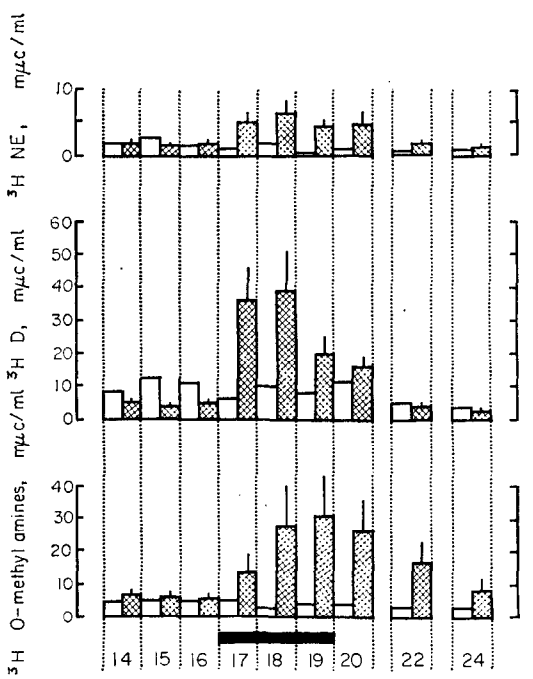

FIG. 6. Effect of *d*-amphetamine on the concentration of ^{3}H-norepinephrine, ^{3}H-dopamine and ^{3}H-*O*-methyl amine metabolites in the perfusion effluent after injection of ^{3}H-dopamine into the left lateral ventricle. The height of each bar represents the mean concentration (vertical lines denote 1 standard error) of ^{3}H-norepinephrine (^{3}H NE), ^{3}H-dopamine (^{3}H D) and ^{3}H-*O*-methyl amines in effluent collected over a 10-min period. In two cats (open bars) the brains were perfused only with artificial cerebrospinal fluid 2 hr after the injection of 15 μc of ^{3}H-dopamine into the left lateral ventricle. In 5 cats (shaded bars) the brains were perfused in a similar manner, except during the time period indicated by the solid horizontal bar below the graph when *d*-amphetamine SO_4 (50 μg/ml) was added to the perfusing fluid.

injection experiments, there was an increase in the concentration of *O*-methyl amine metabolites. Although the compounds in this chromatographic fraction were not specifically identified, they probably represent ^{3}H-normetanephrine and ^{3}H-3-methoxytyramine. The actual concentrations of these compounds remaining in various brain regions are listed in Table 4. ^{3}H-norepinephrine constituted a very small percentage of total amines in the caudate nucleus, compared with other areas.

Effect of intravenous administration of d-*amphetamine on the concentration of ^{3}H-norepinephrine and ^{3}H-normetanephrine in the perfusion effluent.* The time course

TABLE 4. CONCENTRATIONS OF ^{3}H-NOREPINEPHRINE, ^{3}H-DOPAMINE AND *O*-METHLY AMINE METABOLITES IN CAT BRAIN AFTER INTRAVENTRICULAR INJECTION OF ^{3}H-DOPAMINE

Brain region	^{3}H-norepinephrine (mμc/g $\pm$ S.E.)	^{3}H-dopamine (mμc/g $\pm$ S.E.)	^{3}H-*O*-methyl amines (mμc/g $\pm$ S.E.)	Per cent of total amines as ^{3}H-norepinephrine
Left caudate nucleus	124 $\pm$ 34	1791 $\pm$ 458	438 $\pm$ 159	5·3
Hypothalamus	78 $\pm$ 29	55 $\pm$ 24	30 $\pm$ 14	47·9
Septal area	142 $\pm$ 51	183 $\pm$ 64	107 $\pm$ 28	32·9

Two hr after the injection of 15 μc of ^{3}H-dopamine into the left lateral ventricle, the lateral and third ventricles were perfused with artificial cerebrospinal fluid for 4 hr. *d*-Amphetamine (50 μg/ml) was perfused during the last 2 hr as described in Fig. 1. Each value represents the mean concentration of ^{3}H-norepinephrine, ^{3}H-dopamine or ^{3}H-*O*-methyl amines in the brain region as determined in five animals.

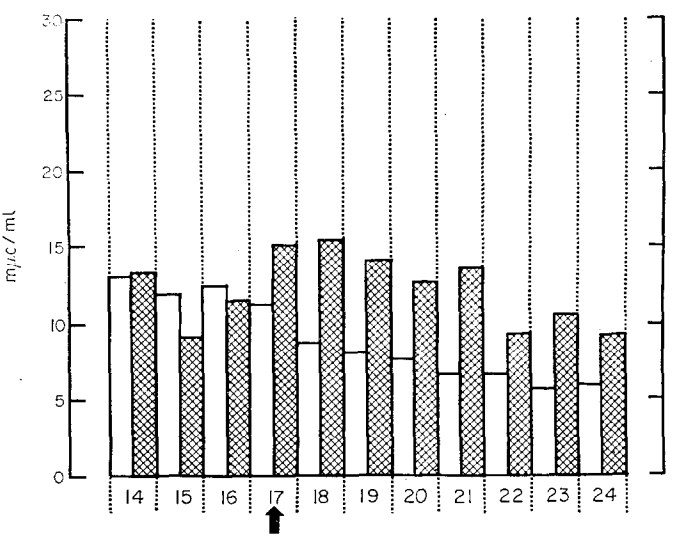

FIG. 7. Effect of an intravenous injection of *d*-amphetamine on the concentration of ^{3}H-norepinephrine in cerebroventricular effluent. One hr after an injection of 5 μc of ^{3}H-norepinephrine into the left lateral ventricle, the lateral and third cerebral ventricles were perfused with artificial cerebrospinal fluid. The height of each bar represents the concentration of ^{3}H-norepinephrine in effluent collected over a 10-min period. The open bars represent samples collected during an experiment in which only artificial cerebrospinal fluid was perfused. The shaded bars represent samples collected during an experiment in which *d*-amphetamine (1 mg/kg) was injected intravenously at the beginning of sample no. 17.

of the effect of an intravenous injection of *d*-amphetamine (1 mg/kg) on the effluent concentration of ^{3}H-norepinephrine is compared with a control (no drug) experiment in Fig. 7. When no drug was given, the concentration of ^{3}H-norepinephrine declined slowly. When *d*-amphetamine was injected, the effluent concentration of ^{3}H-norepinephrine remained constant or increased slightly. A summary of experiments involving the intravenous administration of *d*-amphetamine is presented in Table 5. The effluent concentration of ^{3}H-norepinephrine but not ^{3}H-normetanephrine was significantly higher ($P < 0.01$) in the 30-min period after the injection of *d*-amphetamine than during the same time period in the control experiments.

TABLE 5. EFFECT OF INTRAVENOUS INJECTION OF *d*-AMPHETAMINE ON THE CONCENTRATION OF ^{3}H-NOREPINEPHRINE AND ^{3}H-NORMETANEPHRINE IN THE PERFUSION EFFLUENT*

	N†	^{3}H-norepinephrine (% ± S.E.)	^{3}H-normetanephrine (% ± S.E.)
Control	5	71·7 ± 6·3	75·1 ± 9·1
d-Amphetamine	3	109·7 ± 11·0‡	90·9 ± 4·7

*One hr after an injection of ^{3}H-norepinephrine into the left lateral ventricle, *d*-amphetamine was injected intravenously (1 mg/kg) through a femoral vein at the beginning of sample no. 17 (see Fig. 1). Each value is the mean amount of ^{3}H-norepinephrine (sample nos. 17–19) and ^{3}H-normetanephrine (sample nos. 18–20) represented as the percentage of the amine collected during the 3 predrug samples (14–16).
† = Number of animals.
‡Significantly greater than control ($P < 0.01$).

DISCUSSION

A variety of techniques have been utilized in order to examine the actions of drugs, electrical stimuli, and environmental factors on the properties of suspected neurotransmitters in the brain. Although results of these studies have added greatly to our understanding of neurotransmitters, only limited information can be obtained by observing the brain contents of these substances at fixed points in time. In order to obtain a more dynamic picture of the factors which control the turnover, release, and metabolism of neurotransmitters, efforts have been directed toward continuously monitoring their release from the brain. Some of the techniques that have been used include: (1) affixing collecting cups on the cortex of the brain,[23, 24] (2) implanting push–pull cannulae into various regions of the brain,[6–8] and (3) perfusing the cerebroventricular system.[11] Because most noradrenergic and dopaminergic neurons are not located near the cortical surface of the brain, the first method, although effective for detecting release of acetylcholine,[24] is not useful for studying catecholamine release. Although tissues lying deep within the brain can be effectively perfused with the aid of a push–pull cannula, there have been objections raised to results obtained with this method.[9, 10] Regions which contain high concentrations of norepinephrine (hypothalamus, septum) and dopamine (caudate nucleus) lie adjacent to the cerebroventricular system and they will accumulate catecholamines that are introduced into the ventricular system.[21] It is not unreasonable then to expect that amines from these regions may be released into the cerebrospinal fluid after appropriate stimulation.

Indeed, certain products of catecholamine metabolism have been identified in the cerebrospinal fluid,[25, 26] and the cerebroventricular perfusion technique has proven useful for studying the efflux of acetylcholine,[27] 5-hydroxytryptamine,[28] dopamine, and homovanillic acid[29] from the brain *in situ*.

Primarily because of limitations imposed by the lack of sensitivity of available analytical methods, there have been no reports concerning the release of endogenous norepinephrine from brain *in situ*. However, studies in the rat[30, 31] and cat[21] have indicated that regions which contain the highest amounts of endogenous catecholamines actively accumulate ^{3}H-norepinephrine when it is given into the cerebroventricular system. It has now been demonstrated that drugs and electrical stimuli can release labeled catecholamines and their metabolites when these regions are perfused with the aid of a push–pull cannula[7, 8] or when the ventricular is system perfused.[17, 18, 32]

It has been suggested that amphetamine produces its central nervous system stimulating effects by releasing norepinephrine from nerve terminals in the brain or by blocking the reuptake of this amine into the terminals and thereby increasing the concentration of the amine at receptor sites.[15] Indirect evidence in support of this hypothesis include reports that: large doses of amphetamines lower the endogenous content of norepinephrine in brain;[13, 14] amphetamine releases ^{3}H-norepinephrine from brain slices and reduces the fluorescent intensity in noradrenergic neurons;[2] blockade of catecholamine synthesis by α-methyltyrosine blocks central stimulant actions of amphetamine;[33] and amphetamine blocks accumulation of intraventricularly administered ^{3}H-norepinephrine[34] and increases the content of ^{3}H-normetanephrine in brain.[35]

More direct evidence that amphetamine may affect catecholamine release or reuptake by the central nervous system has recently been offered by studies which demonstrate an increase in the concentration of ^{3}H-norepinephrine in the perfusion effluent after intraperitoneal[8] or intraventricular administration of *d*-amphetamine.[17] The results of the present study indicate that very small amounts of *d*-amphetamine (as low as 25 μg/ml) can significantly increase the concentration of ^{3}H-norepinephrine in the perfusion effluent, whereas much larger amounts (500 μg/ml) were required to demonstrate release of endogenous dopamine from the caudate nucleus using a push–pull cannula.[16] The methods used in the present study thus allow detection of a very small increase in the efflux of norepinephrine, avoid the administration of large drug concentrations at small perfusion sites, and cause minimal damage to the region perfused.

Some indication of how amphetamine acts to increase the effluent concentrations of ^{3}H-norepinephrine and ^{3}H-normetanephrine may be obtained by examining the time course of the drug's action. For example, it has been suggested that some of the effects of amphetamine are mediated by one of its metabolites, *p*-hydroxynorephedrine.[36] However, the immediate increase in the concentration of ^{3}H-norepinephrine in the effluent suggests that this effect is mediated by amphetamine *per se* and not by a metabolite. The delayed increased in the concentration of ^{3}H-normetanephrine suggests that amphetamine releases ^{3}H-norepinephrine from catecholamine-containing neurons and some of it is subsequently metabolized by extraneuronal catecholamine-*O*-methyltransferase. With the present technique, however, it is not possible to determine if the increased efflux of ^{3}H-norepinephrine is secondary to an amphetamine-induced release from the nerve terminal or if it is because of a blockade of reuptake

into the neuron. In the present study it was demonstrated that at least at one dose level (50 μg/ml) the *d*-isomer significantly increased the effluent concentrations of ^{3}H-norepinephrine and ^{3}H-normetanephrine, whereas the *l*-isomer did not. Thus the relative abilities of these two isomers to increase the efflux of ^{3}H-norepinephrine and ^{3}H-normetanephrine coincide with their relative abilities to stimulate the central nervous system[15] and to lower steady-state brain norepinephrine concentrations.[37]

In order to determine the site at which amphetamine acts to increase the efflux of tritiated amines, ^{3}H-norepinephrine was injected into various sections of the ventricular system. The greatest efflux occurred when ^{3}H-norepinephrine was injected into the lateral ventricle, suggesting that the primary site of release must border the lateral ventricle. Since the caudate nucleus comprises a large portion of the surface area in the lateral ventricle and it has the capacity to accumulate ^{3}H-norepinephrine[21], this structure appears to be the most likely source of the released amines. The preferential release of ^{3}H-dopamine after injection of this amine into the lateral ventricle also points to the caudate nucleus as one site of the actions of amphetamine. Since there was very little formation of ^{3}H-norepinephrine from ^{3}H-dopamine in the caudate nucleus (Table 4), the greater efflux of dopamine probably occurred because there was a greater concentration of ^{3}H-dopamine than ^{3}H-norepinephrine in this area.

The most significant result of the dopamine injection experiments was the finding that *d*-amphetamine released a small amount of ^{3}H-norepinephrine formed *in situ* from ^{3}H-dopamine. Since dopamine-β-hydroxylase, the enzyme which catalyzes the formation of norepinephrine from dopamine, appears to be located in storage granules of noradrenergic neurons in the brain,[38] the data strongly suggest that the action of amphetamine to increase the efflux of labeled amines is the result of specific actions on catecholamine-containing neurons. This specificity of the actions of amphetamine on catecholamine release was further substantiated by the experiments with ^{14}C-inulin and ^{14}C-urea. In a previous study,[9] utilizing a push-pull cannulation technique, it was demonstrated that stimuli which increased the efflux of ^{3}H-norepinephrine also increased the efflux of metabolically inert substances (urea, inulin). The authors indicated that better controls are needed before data obtained with push-pull cannulae can be interpreted as evidence for the release of suspected neurotransmitters. In the present study amphetamine did not alter the efflux of ^{14}C-inulin or ^{14}C-urea.

Although it was shown that amphetamine can increase the efflux from brain tissues when the drug is perfused through the ventricular system, this action may not be related to the drug's pharmacological effects when it is given systemically. However, when *d*-amphetamine was injected intravenously, a significant increase in the efflux of ^{3}H-norepinephrine occurred. This result further indicates the specificity of amphetamine's action since it is unlikely that there was a sufficient concentration of the drug in the ventricular system to evoke a nonspecific exchange with ^{3}H-norepinephrine at an extraneuronal binding site. The intravenous injection experiments also indicate that the cerebroventricular perfusion technique is useful for monitoring the effects of systemically administered drugs on brain catecholamine release, as well as when they are perfused through the ventricular system.

Although the actual brain area or areas at which *d*-amphetamine acts to increase the efflux of norepinephrine has not been conclusively established in the present study,

it is clear that the drug can release labeled norepinephrine from neuronal sites near the ventricular system and the data suggests that amphetamine may exert its behavioral effects by releasing and/or blocking the reuptake of catecholamines from noradrenergic or dopaminergic nerve terminals.

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REFERENCES

1. L. L. IVERSEN, in *The Uptake and Storage of Noradrenaline in Sympathetic Nerves*, p. 244 Cambridge Univ. Press, Cambridge (1967).
2. A. CARLSSON, M. LINDQVIST, A. DAHLSTROM, K. FUXE and D. MASUOKA, *J. Pharm. Pharmac.* **17**, 521 (1965).
3. R. J. BALDESSARINI and I. J. KOPIN, *J. Pharmac. exp. Ther.* **156**, 31 (1967).
4. R. J. BALDESSARINI and I. J. KOPIN, *Science, N. Y.* **152**, 1630 (1966).
5. N. -E. ANDEN, A. CARLSSON, N. -A. HILLARP and T. MAGNUSSON, *Life Sci.* **3**, 523 (1964).
6. H. MCLENNAN, *J. Physiol., Lond.* **174**, 152 (1964).
7. F. SULSER and J. V. DINGELL, *Agressologie* **9**, 281 (1968).
8. L. STEIN and C. D. WISE, *J. comp. physiol. Psychol.* **67**, 189 (1969).
9. T. N. CHASE and I. J. KOPIN, *Nature, Lond.* **217**, 466 (1968).
10. F. E. BLOOM and N. J. GIARMAN, *Ann. Rev. Pharmac.* **8**, 229 (1968).
11. E. A. CARMICHAEL, W. FELDBERG and K. FLEISCHHAUER, *J. Physiol., Lond.* **173**, 354 (1964).
12. J. H. BURN and M. J. RAND, *J. Physiol., Lond.* **144**, 314 (1958).
13. S. SANAN and M. VOGT, *Br. J. Pharmac. Chemother.* **18**, 109 (1962).
14. K. E. MOORE and E. W. LARIVIERE, *Biochem. Pharmac.* **12**, 1283 (1963).
15. L. STEIN, *Fedn Proc.* **32**, 836 (1964).
16. G. M. MCKENZIE and J. C. SZERB, *J. Pharmac. exp. Ther.* **162**, 302 (1968).
17. L. A. CARR and K. E. MOORE, *Science, N. Y.* **164**, 322 (1969).
18. L. A. CARR and K. E. MOORE, *Fedn Proc.* **28**, 796 (1969).
19. L. E. MCCARTHY and H. L. BORISON, *Anat. Rec.* **155**, 305 (1966).
20. J. R. PAPPENHEIMER, S. R. HEISEY, E. F. JORDAN and J. DE C. DOWNER, *Am. J. Physiol.* **203**, 763 (1962).
21. L. A. CARR and K. E. MOORE, *Biochem. Pharmac.* **18**, 1907 (1969).
22. R. G. D. STEEL and J. H. TORRIE, in *Principles and Procedures of Statistics*, McGraw-Hill, New York (1960).
23. F. C. MACINTOSH and P. E. OBORIN, *Abst. XIX Int. Physiol. Congr.* 580 (1953).
24. J. F. MITCHELL, *J. Physiol., Lond.* **165**, 98 (1963).
25. N. -E. ANDEN, B. -E. ROOS and B. WERDINIUS, *Experientia* **19**, 359 (1963).
26. G. W. ASHCROFT, T. B. B. CRAWFORD, R. D. DOW and H. C. GULDBERG, *Br. J. Pharmac. Chemother.* **33**, 441 (1968).
27. D. BELESIN, E. A. CARMICHAEL and W. FELDBERG, *J. Physiol., Lond.* **173**, 368 (1964).
28. W. FELDBERG and R. D. MYERS, *J. Physiol., Lond.* **184**, 837 (1966).
29. P. J. PORTIG, D. F. SHARMAN and M. VOGT, *J. Physiol., Lond.* **194**, 565 (1968).
30. J. GLOWINSKI, I. J. KOPIN and J. AXELROD, *J. Neurochem.* **12**, 25 (1965).
31. J. GLOWINSKI and R. J. BALDESSARINI, *Pharmac. Rev.* **18**, 1201 (1966).
32. D. PALAIC and P. A. KHAIRALLAH, *J. Neurochem.* **15**, 1195 (1968).
33. A. WEISSMAN, B. K. KOE and S. S. TENEN, *J. Pharmac. exp. Ther.* **151**, 339 (1966).
34. J. GLOWINSKI and J. AXELROD, *J. Pharmac. Exp. Ther.* **149**, 43 (1965).
35. J. GLOWINSKI, J. AXELROD and L. IVERSEN, *J. Pharmac. exp. Ther.* **153**, 30 (1966).
36. A. GROPPETTI and E. COSTA, *Fedn Proc.* **28**, 795 (1969).
37. K. E. MOORE, *J. Pharmac. exp. Ther.* **142**, 6 (1963).
38. S. UDENFRIEND, in *Mechanisms of Release of Biogenic Amines*, (Eds. U. S. VON EULER, S. ROSELL and B. UVNAS) pp. 103–108. Pergamon Press, Oxford (1967).