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SELECTIVITY OF RELEASE OF NOREPINEPHRINE, DOPAMINE AND 5-HYDROXYTRYPTAMINE BY AMPHETAMINE IN VARIOUS REGIONS OF RAT BRAIN*

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Abstract—Tissues from various regions of rat brain were incubated with [^{3}H]norepinephrine, [^{3}H]dopamine or [^{3}H]5-hydroxytryptamine; excess amine was washed from the tissues and the [^{3}H]amines present in the incubation medium and tissues were measured. The release of the accumulated [^{3}H]amine by amphetamine was related to the nature of the neurons in the several regions and the release of each of the [^{3}H]amines was quite selective for neurons which contain the corresponding endogenous amine. The release of [^{3}H]norepinephrine from norepinephrine neurons is most sensitive to amphetamine, while the release of [^{3}H]dopamine from dopamine neurons requires higher concentrations of amphetamine. Release of [^{3}H]5-hydroxytryptamine from 5-hydroxytryptamine neurons appears to be least sensitive to amphetamine. This selective effect of amphetamine on neurons containing biogenic amines may help to explain the behavioral changes which occur at different doses of amphetamine. The selectivity of release of the [^{3}H]-amines was also examined by observing the accumulation and release of [^{3}H]norepinephrine and [^{3}H]5-hydroxytryptamine in the presence of five-fold higher concentrations of the unlabeled amines. There was relatively little effect of unlabeled norepinephrine on the accumulation and release of [^{3}H]5-hydroxytryptamine or of unlabeled 5-hydroxytryptamine on [^{3}H]norepinephrine accumulation and release. Unlabeled dopamine reduced the accumulation and altered the release of [^{3}H]norepinephrine and [^{3}H]5-hydroxytryptamine, but this was much less marked than the effect of the unlabeled analogues of the [^{3}H]amines. The release of each of the [^{3}H]amines by its unlabeled analogue occurred with concentrations as low as, or lower than, the release produced by amphetamine or the other unlabeled amines. The release of the [^{3}H]amines by a five-fold higher concentration of the unlabeled analogues was so high that it was not increased further by very high concentrations of amphetamine (10^{-3} M). These results suggest that, under the conditions of this study, the [^{3}H]amines are accumulated within specific neurons of brain tissue and that they are selectively released by amphetamine.

IT HAS BEEN postulated that the behavioral effects of amphetamine are due to release of neurotransmitters from nerve endings in brain, resulting in increased concentrations of the biogenic amines interacting with the receptors of the postsynaptic nerve cells.[1,2] The release of biogenic amines by amphetamine occurs *in vitro*[3–5] as well as in the intact animal.[6–8] That is, if isolated brain tissue has accumulated [^{3}H]norepinephrine ([^{3}H]-NE), [^{3}H]dopamine ([^{3}H]-DA) or [^{3}H]5-hydroxytryptamine ([^{3}H]-5-HT),

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subsequent addition of amphetamine results in a net efflux of the three [^{3}H]amines from the tissue into the incubation medium.[4,5,9]

There is evidence which suggests that the three [^{3}H]amines are not uniformly released by amphetamine. For example, when cerebral cortex had accumulated either [^{3}H]NE, [^{3}H]DA or [^{3}H]5-HT, release of [^{3}H]NE was observed at very low concentrations of amphetamine, while higher concentrations were required to release [^{3}H]DA and [^{3}H]5-HT.[9–13] In the present study, the release of [^{3}H]NE, [^{3}H]DA and [^{3}H]5-HT by amphetamine is related to the diverse distribution of neurons containing the amines in three regions of rat brain. The selectivity of release is further examined by comparing the displacement of [^{3}H]amine by unlabeled amine with the efflux of [^{3}H]amine produced by amphetamine.

METHODS

Release of [^{3}H]amines from isolated brain tissue was studied by incubating the tissue with [^{3}H]amine, allowing the [^{3}H]amine to be taken up into the neurons, and observing the amount of [^{3}H]amine in the incubation medium after incubation with amphetamine or unlabeled biogenic amine. A detailed description of this procedure has been previously presented.[5,9,13] Adult male Sprague–Dawley rats (200–300 g) were killed by decapitation and their brains were removed. The cerebral cortex (white matter) removed, the corpus striatum and medulla oblongata were dissected and chopped at 0·3-mm intervals with a mechanical tissue chopper. The tissue from one rat was sufficient for each experiment with cerebral cortex. When the corpus striatum and medulla oblongata were used, the tissues from three rats were combined. The chopped tissue was incubated at 37° for 10 min in a physiological medium prior to addition of the [^{3}H]amine. *dl*-NE-7-[^{3}H] (8 μCi), DA ring-labeled-[^{3}H] (0·6 μCi) or 5-HT ring-labeled-[^{3}H] (17 μCi) was added to the suspension to give a final concentration of 10^{-6} M. The tissue was incubated with [^{3}H]amine for 15 min and the tissue was separated from the incubation medium by centrifugation. The tissue was then washed with fresh incubation medium three times followed by a 20-min incubation (37°) in fresh medium. The tissue suspension was then divided into six fractions and each fraction was incubated for 30 min at 37° with one of several concentrations of amphetamine or unlabeled NE, DA or 5-HT. In some experiments, unlabeled 5×10^{-6} M NE, DA or 5-HT was present either throughout the incubation procedure or only during the 15-min incubation with the [^{3}H]amine.

After the 30-min incubation with the releasing substance, the medium was separated from the tissue by centrifugation. The incubation medium was acidified to pH 1 with HCl and the tissue was homogenized in 2 N HCl. DA, NE, or 5-HT (100 μg each) was added to the medium and tissue samples prior to homogenization as carrier substances and, in the cases of DA and NE, for determination of recoveries. The precipitated protein in each fraction was separated from the extract by centrifugation. [^{3}H]NE and [^{3}H]DA in the medium and tissue extracts were separated from their deaminated metabolites by adsorption on (pH 6·5) and elution from Dowex 50, Na^+-form, with 1 N HCl and 2 N HCl respectively. The effluent from the Dowex column represents primarily deaminated metabolites,[14] while the eluate represents primarily catecholamines. *O*-methylated amines represent less than 10 per cent of the total amines eluted from the column.[9] The recoveries from each sample were determined by spectrofluorometric measurement (activation at 285 nm and emission at

335 nm) of the amines in the eluate from Dowex columns. The recoveries ranged from 85 to 90 per cent. The radioactivity in each fraction was determined by liquid scintillation spectrometry. The radioactivity in each sample was corrected for recovery.

[^{3}H]5-HT was separated from its prinicipal [^{3}H]-deaminated metabolite (5-hydroxyindoleacetic acid) by a method described by Udenfriend *et al.*[15] The medium and tissue extracts were prepared as described above. The acidified extracts were saturated with NaCl and added to tubes containing 20 ml diethyl ether. The tubes were shaken for 15 min and centrifuged. The radioactivity from each phase was determined by liquid scintillation spectrometry. The ether phase contained 5-hydroxyindoleacetic acid and the aqueous phase contained 5-HT. When [^{3}H]5-HT was added to acidified tissue extracts, 92–98 per cent remained in the aqueous phase. Fluorometric analysis of 5-hydroxyindoleacetic acid[15] revealed that approximately 100 per cent of this substance was extracted into ether. Since the recoveries of both substances were essentially complete, recovery determination was omitted with this assay.

The protein content per sample was determined by the Biuret method[16] in each sample, and both medium and tissue radioactivities were based on the amount of tissue (mg protein) present in the sample. Protein contents from samples of the three tissues were: (1) cerebral cortex, 11·1 $\pm$ 0·3 (mean $\pm$ S.E.M.) mg/sample; (2) corpus striatum, 3·2 $\pm$ 0·1 mg/sample; and (3) medulla oblongata, 5·5 $\pm$ 0·2 mg/sample. The results were generally expressed as a percentage of [^{3}H]amine in the incubation medium calculated as

$$\frac{[^3\text{H}]\text{amine in medium} \times 100}{([^3\text{H}]\text{amine in medium}) + ([^3\text{H}]\text{amine in tissue})}.$$

Statistical comparisons were made by Student's *t*-test.

The ED_{50} (median effective dose) was estimated as the concentration of drug which gave half the maximal response (adjusted for "spontaneous release"). This approximation is probably close to the real value, since in most cases either the concentration–effect curve reached a plateau or a maximal response was obtained.

Substances. Equivalents of NE, DA, 5-HT and amphetamine were used in the calculation of concentrations. l-Arterenol (NE)-D-bitartrate was obtained from Mann Research Laboratories, New York, N.Y. 3-Hydroxytryramine (DA) HCl was obtained from Calbiochem, La Jolla, Calif. 5-HT creatinine sulfate was obtained from Sigma Chemical Co., St. Louis, Mo. D-Amphetamine sulfate was obtained from Smith, Kline & French Laboratories, Philadelphia, Pa. *dl*-NE-7-[^{3}H]hydrochloride (3·8 Ci/m-mole), DA ring-labeled-[^{3}H]hydrochloride (0·3 Ci/m-mole) and 5-HT ring-labeled-[^{3}H]creatinine sulfate monohydrate (8·5 Ci/m-mole) were obtained from Amersham/Searle Corp., Arlington Heights, Ill.

RESULTS

Release of [^{3}H]NE, [^{3}H]DA and [^{3}H]5-HT from isolated brain tissue by amphetamine. The release of each of the [^{3}H]amines from cerebral cortex in the absence of amphetamine was markedly different (Fig. 1). This control or "spontaneous" release was high for [^{3}H]5-HT, less for [^{3}H]NE, and least for [^{3}H]DA. Incubation with amphetamine resulted in release of each of the [^{3}H]amines into the incubation medium, but the concentration–effect curves for the three amines were different. [^{3}H]NE was released by

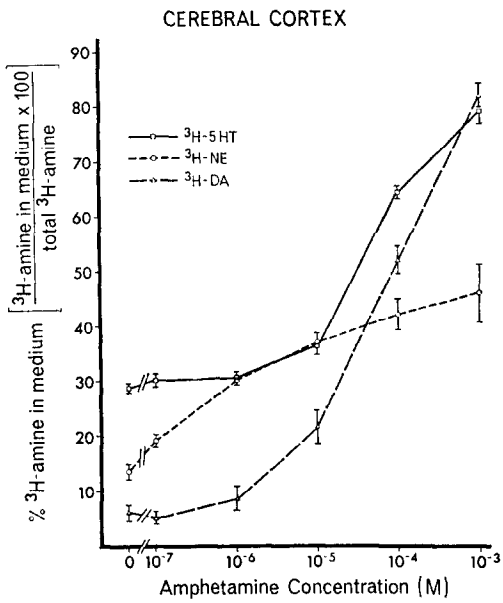

FIG. 1. Amphetamine-induced release of [³H]NE, [³H]DA and [³H]5-HT from rat cerebral cortex. Chopped cerebral cortex which had accumulated [³H]amine was incubated with various concentrations of *d*-amphetamine. The [³H]amine was separated from the deaminated metabolites and the proportion of the [³H]amine which appeared in the incubation medium was used as an indication of release of [³H]amine from the tissue. Each value is the mean ± S.E.M. of four to eight experiments

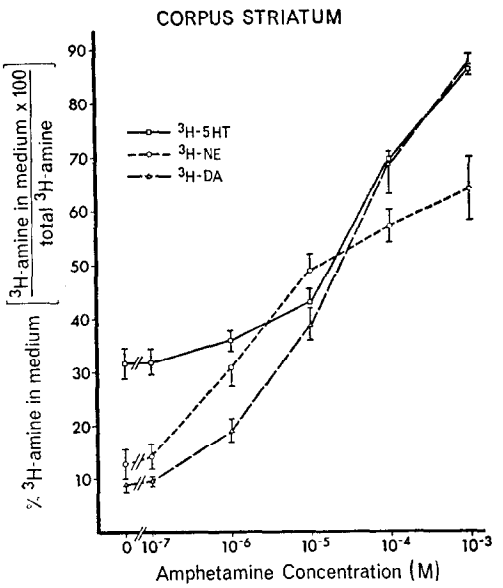

FIG. 2. Amphetamine-induced release of [³H]NE, [³H]DA and [³H]5-HT from rat corpus striatum. For description of experimental conditions, see Fig. 1. Each value is the mean ± S.E.M. of four to seven experiments.

10^{-7} M amphetamine ($P < 0.01$), while 10^{-5} M amphetamine was required to release [^{3}H]DA ($P < 0.02$) and [^{3}H]5-HT ($P < 0.01$). The ED_{50} values for release from cerebral cortex were: NE, 10^{-6} M; DA, 5.4×10^{-5} M; and 5-HT, 4.3×10^{-5} M. At high concentrations of amphetamine (10^{-4} to 10^{-3} M), the proportion of [^{3}H]DA and [^{3}H]5-HT released from the tissue was greater than that of [^{3}H]NE.

Similar observations were made when the experiments were conducted with medulla oblongata (not shown). However, in this case, significant release of all three labeled amines was achieved only with concentrations of amphetamine of 10^{-6} M or higher. The ED_{50} values for release from medulla oblongata were: NE, 1.8×10^{-6} M; DA, 6.7×10^{-5} M; and 5-HT, 6.3×10^{-5} M.

The "spontaneous" release of [^{3}H]DA and [^{3}H]NE from corpus striatum was approximately the same and was much less than the "spontaneous" release of [^{3}H]-5-HT (Fig. 2). In contrast to the other tissues, release of [^{3}H]DA from corpus striatum was increased by 10^{-6} M amphetamine ($P < 0.05$). The ED_{50} values for release from corpus striatum were: NE, 2.7×10^{-6} M; DA, 5×10^{-5} M; and 5-HT, 4.3×10^{-5} M. The release of [^{3}H]NE and [^{3}H]DA by amphetamine was quite similar at low concentrations of amphetamine (10^{-6} to 10^{-5} M). At higher amphetamine concentrations (10^{-4} to 10^{-3} M), the concentration–effect curve for release of [^{3}H]DA was very similar to that for release of [^{3}H]5-HT.

The accumulation of each of the [^{3}H]amines in the release experiments was calculated as the sum of the [^{3}H]amines and [^{3}H]deaminated metabolites in the medium and tissue fractions (Table 1). The accumulation of [^{3}H]5-HT in the cerebral cortex

TABLE 1. ACCUMULATION OF [^{3}H]NE, [^{3}H]DA AND [^{3}H]5-HT DURING RELEASE EXPERIMENTS*

Tissue	[^{3}H]NE	[^{3}H]DA	[^{3}H]5-HT
Cerebral cortex	2.6 ± 0.4	2.7 ± 0.1	3.5 ± 0.3
Medulla oblongata	5.9 ± 0.1	3.1 ± 0.2	8.7 ± 1.3
Corpus striatum	5.4 ± 0.5	18.3 ± 2.7	14.6 ± 1.2

* Tissue from various regions of rat brain were incubated with 10^{-6} M of either [^{3}H]NE, [^{3}H]DA or [^{3}H]5-HT. The tissue was washed and incubated for an additional 30 min. The dis/min/mg protein of [^{3}H]metabolites in the tissue and medium were totaled and converted to p-moles. Thus, each value represents the amine equivalent in p-moles/mg protein in each of the three regions and is the mean ± S.E.M. of four to eight determinations.

was slightly greater ($P < 0.05$) than that of [^{3}H]DA and [^{3}H]NE. In medulla oblongata, [^{3}H]5-HT accumulated to the greatest degree and [^{3}H]DA accumulated in the tissue to the smallest extent. This was in contrast to the corpus striatum in which the accumulation of [^{3}H]DA was the highest, [^{3}H]5-HT accumulated less, and the accumulation of [^{3}H]NE was least. [^{3}H]5-HT and [^{3}H]DA were taken up and retained to a much greater degree in the corpus striatum than in the other tissues.

Effect of unlabeled biogenic amines on the release of [^{3}H]5-HT from medulla oblongata and corpus striatum. The specificity of uptake, storage and release of [^{3}H]5-HT was examined by incubating medulla oblongata with unlabeled 5×10^{-6} M NE, DA or

5-HT concomitant with 10^{-6} M [^{3}H]5-HT. The [^{3}H]5-HT was present only during the intial uptake portion of the experiment, while the unlabeled amines were present during the washing and release phases as well. The presence of unlabeled 5-HT and DA throughout the entire incubation procedure was associated with an increase in the proportion of [^{3}H]5-HT in the incubation medium (Fig. 3). The release produced

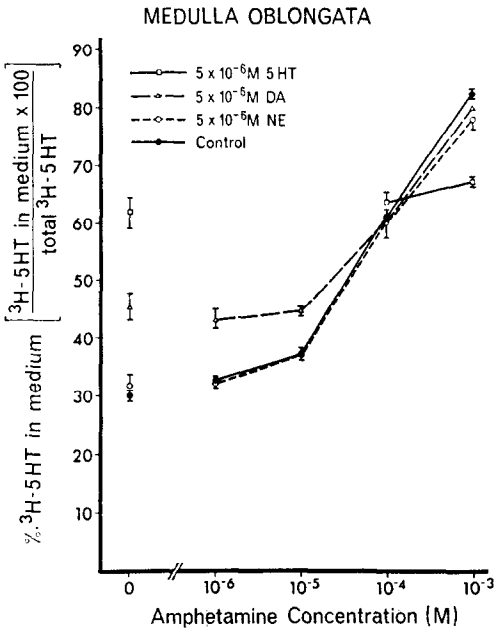

FIG. 3. Effect of unlabeled 5-HT, DA and NE on the release of [^{3}H]5-HT from medulla oblongata by amphetamine. Chopped medulla oblongata was incubated with [^{3}H]5-HT (10^{-6} M), the excess [^{3}H]5-HT was removed by washing, and the tissue was subsequently incubated with various concentrations of amphetamine. In some experiments, 5×10^{-6} M 5-HT, DA or NE was present throughout the entire incubation procedure. Total radioactivity present in each sample represents: 8·4 ± 0·9 p-moles/mg protein of [^{3}H]5-HT in control, 9·6 ± 0·6 p-moles/mg protein with 5×10^{-6} M DA, 7·7 ± 1·1 p-moles/mg protein with NE, and 4·9 ± 0·3 p-moles/mg protein with 5×10^{-6} M 5-HT. Each value represents the mean ± S.E.M. of four to eight experiments.

by 5-HT was much greater than that produced by DA. The efflux of [^{3}H]5-HT was not altered by unlabeled NE. Release of [^{3}H]5-HT by amphetamine was affected by unlabeled DA only at the lower amphetamine concentrations (10^{-6} to 10^{-5} M). The marked increase in the efflux of [^{3}H]5-HT caused by unlabeled 5-HT was not further increased by amphetamine. The uptake and retention of [^{3}H]5-HT were inhibited by unlabeled 5-HT ($P < 0.05$) and were not affected by unlabeled DA or NE (Fig. 3, legend).

Similar effects were observed in the corpus striatum (not shown). In contrast to experiments with medulla oblongata, the effects of unlabeled NE and DA on the efflux of [^{3}H]5-HT from corpus striatum were quite similar.

Effect of unlabeled biogenic amines on the release of [^{3}H]NE from cerebral cortex. The presence of 5×10^{-6} M NE throughout the incubation procedure resulted in a marked ($P < 0.001$) increase in the proportion of [^{3}H]NE in the incubation medium (Fig. 4). Even large concentrations of amphetamine (10^{-3} M) did not further increase

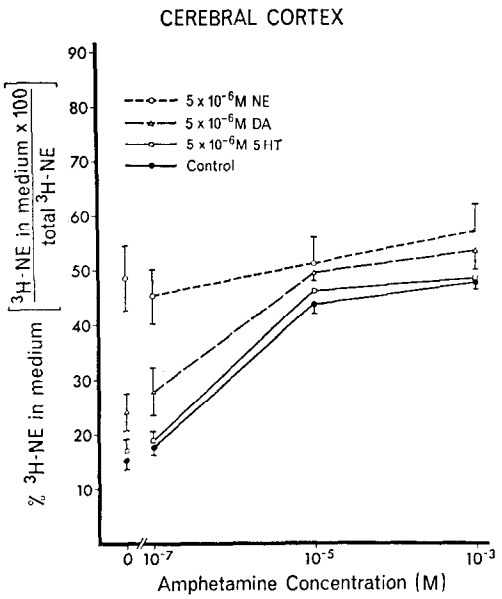

FIG. 4. Effect of unlabeled NE, DA and 5-HT on the release of [^{3}H]NE from cerebral cortex by amphetamine. The experimental conditions are similar to those described in Fig. 3. Total radioactivity in each sample represents: 2·6 ± 0·3 p-moles/mg protein of [^{3}H]NE in control samples, 1·6 ± 0·2 p-moles/mg protein with 5×10^{-6} M DA, 2·8 ± 0·2 p-moles/mg protein with 5×10^{-6} M 5-HT and 1·2 ± 0·1 p-moles/mg protein with 5×10^{-6} M NE. Each value represents the mean ± S.E.M. of four to eight experiments.

this release. DA (5×10^{-6} M) also increased the [^{3}H]NE in the incubation medium ($P < 0.05$), but this was less marked than the effect of unlabeled NE. Unlabeled 5-HT (5×10^{-6} M) had no effect on the release of [^{3}H]NE by amphetamine. The total radioactivity representing [^{3}H]NE was reduced by DA ($P < 0.05$) and NE ($P < 0.01$) but not by 5-HT (Fig. 4, legend).

If the unlabeled amines were present during the uptake of [^{3}H]NE but not during the washing or release phases of the experiment, the release of [^{3}H]NE in the presence and absence of amphetamine was unaffected (not shown).

Release of [^{3}H]NE by NE, DA and amphetamine. When the release of [^{3}H]NE from cerebral cortex by unlabeled NE, DA and amphetamine was studied, it was observed that NE and DA were equipotent in releasing [^{3}H]NE and that both of these amines released a greater proportion of the [^{3}H]NE from the tissue than did amphetamine (Fig. 5). Pargyline (2.5×10^{-4} M) was present throughout the incubation procedure to inhibit monoamine oxidase. Thus, differential deamination of [^{3}H]NE in experiments with the three amines would not explain the differences in their releasing properties.

Release of [^{3}H]DA from corpus striatum by DA, NE and amphetamine. DA was the most potent of the three amines in releasing [^{3}H]DA from corpus striatum (Fig. 6). Low concentrations of NE (10^{-7} to 10^{-5} M) were similar to amphetamine, and high concentrations (10^{-4} to 10^{-3} M) were similar to DA.

Release of [^{3}H]5-HT from corpus striatum by 5-HT, DA, amphetamine and NE. 5-HT was the most potent of the four amines in releasing [^{3}H]5-HT from isolated corpus striatum (Fig. 7). The potencies of the other amines were: DA > amphetamine > NE.

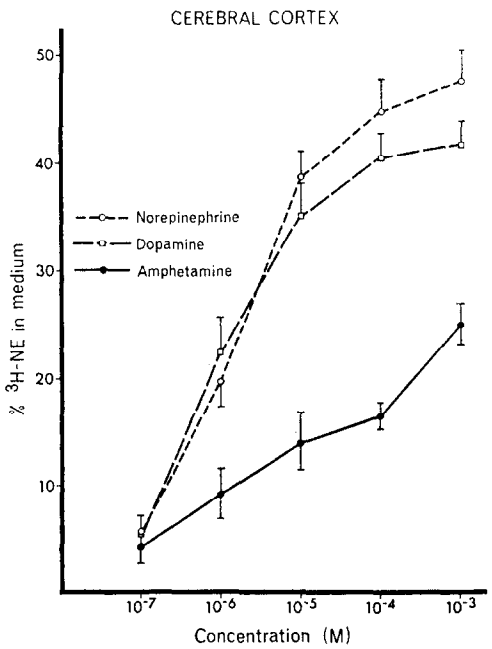

FIG. 5. Comparison of the effects of NE, DA and amphetamine on the release of [^{3}H]NE from cerebral cortex. Chopped rat cerebral cortex was incubated with 10^{-6} M [^{3}H]NE and excess amine was removed from the tissue by washing with fresh incubation medium. The tissue was then incubated with various concentrations of NE, DA and amphetamine. Pargyline, $2{\cdot}5 \times 10^{-4}$ M, was present throughout the entire procedure. [^{3}H]NE in the medium and tissue was isolated by cation-exchange chromatography. Release in the absence of releasing drug was 19·0 ± 1·4 per cent. This value was subtracted from experimental values. Each value represents the mean ± S.E.M. of four experiments.

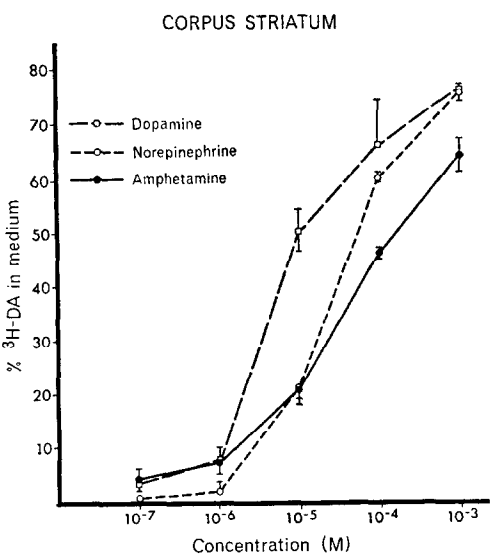

FIG. 6. Comparison of the effects of NE, DA and amphetamine on the release of [^{3}H]DA from corpus striatum. The experimental conditions were similar to those described in Fig. 5. Release in the absence of releasing drug was 8·8 ± 1·1 per cent. This value was subtracted from the experimental values. Each value represents the mean ± S.E.M. of four experiments.

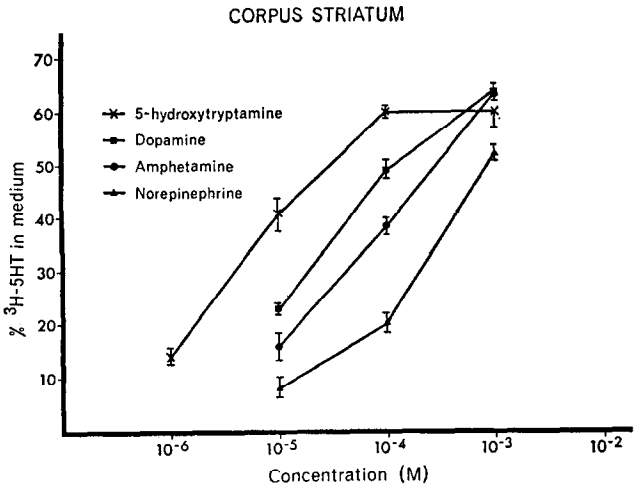

FIG. 7. Comparison of the effects of 5-HT, DA, amphetamine and NE on the release of [^{3}H]5-HT from corpus striatum. The experimental conditions were similar to those described in Fig. 5, except that the [^{3}H]5-HT was isolated by extraction of the metabolites into ether rather than by cation-exchange chromatography. Release in the absence of releasing drug was 22·9 ± 1·0 per cent. This value was subtracted from the experimental values. Each value represents the mean ± S.E.M. of four experiments.

DISCUSSION

The release of [^{3}H]NE from all three brain regions occurred at very low concentrations of amphetamine. The ED_{50} values for release of [^{3}H]NE were 1–3 × 10^{-6} M while those for release of [^{3}H]DA and [^{3}H]5-HT were 10^{-5} M or greater. The release of [^{3}H]NE by amphetamine was somewhat dependent upon the region of brain from which release occurred. Of the three regions studied, the cerebral cortex appeared to be most sensitive to this action of amphetamine, since: (1) the ED_{50} for release from cerebral cortex was lower than the ED_{50} values from corpus striatum and medulla oblongata; and (2) the "threshold" concentration of amphetamine for release from cerebral cortex was 10^{-7} M, while 10^{-6} M was required to release [^{3}H]NE from the other brain regions.

Histochemical studies on the release of NE from neurons in the CNS have also demonstrated that the cerebral cortex is the brain region which is most sensitive to amphetamine.[17] Doses as low as 0·5 mg/kg of amphetamine decrease the fluorescence intensity in nerve endings in cerebral cortex, while 0·75 to 1·0 mg/kg is necessary to release NE from other areas containing NE neurons.

The release of [^{3}H]DA from corpus striatum, which contains a high density of DA nerve endings,[16,18] is also rather selective since the "threshold" concentration of amphetamine for release from corpus striatum is 10^{-6} M, while 10^{-5} M is necessary to release [^{3}H]DA from cerebral cortex, which contains few or no DA neurons.[18–20]

The ED_{50} for release of [^{3}H]5-HT by amphetamine is about the same for the three tissues studied (4·3 to 6·3 × 10^{-5} M). The "threshold" concentration of amphetamine necessary to release [^{3}H]5-HT is 10^{-5} M, from all three tissues. Since all three regions

contain 5-HT neurons, this is again consistent with the release by amphetamine corresponding to the distribution of the neurons.

Interpretation of the selectivity of the effect of amphetamine on the release of [^{3}H]-amines requires an examination of the selectivity of the uptake of the [^{3}H]amines in the various brain regions. Snyder and Coyle[21] investigated the uptake of [^{3}H]NE and [^{3}H]DA in homogenates of various regions of the brain. They found that there was a single K_m for the uptake of [^{3}H]NE into regions containing predominantly NE neurons rather than DA neurons. In the corpus striatum, the K_m for the uptake of [^{3}H]NE was five times higher than that in other neurons. This suggested a lower affinity of NE for uptake into DA neurons. The K_m for the uptake of [^{3}H]DA in the corpus striatum was one-fifth of the K_m for [^{3}H]NE, indicating that [^{3}H]DA has a higher affinity than [^{3}H]NE for the uptake sites of DA neurons. Snyder and Coyle[21] also presented evidence that DA can be taken up by the NE uptake system in NE neurons. Thus, there appears to be some specificity for the uptake of the two [^{3}H]-amines, although there is considerable overlap depending upon the concentration of [^{3}H]amine employed.

Shaskan and Snyder[22] compared the uptake of [^{3}H]NE with that of [^{3}H]5-HT in various regions of rat brain. It appears as if there are two transport systems for 5-HT: a high affinity uptake observed at low concentrations of [^{3}H]5-HT, and a low affinity uptake observed at higher concentrations. Shaskan and Snyder[22] presented evidence that the low affinity uptake system involved uptake into catecholamine-containing neurons.

In the present study, the total accumulation of radioactivity of the [^{3}H]amines in the three brain regions reflects not only initial neuronal uptake but storage and retention of the amines as well, since the measurements were made after a 15-min initial incubation and an extensive washing of the tissue. The accumulation of the [^{3}H]amines correlated to some degree with the density of the corresponding neurons in the three brain regions. The accumulation of [^{3}H]5-HT was highest in the corpus striatum, which contains a high density of 5-HT neurons.[23–25] The accumulation of [^{3}H]NE was greater in medulla oblongata, which contains both cell bodies and nerve endings of NE neurons, than in cerebral cortex, which contains a low density of NE nerve endings. The accumulation of [^{3}H]NE into the corpus striatum was relatively high and may represent the accumulation of [^{3}H]NE into DA neurons. However, this accumulation of [^{3}H]NE was much less than that of either [^{3}H]DA or [^{3}H]5-HT. The uptake and retention of [^{3}H]DA were much higher in the corpus striatum, which contains a high density of DA nerve endings, than in medulla oblongata or cerebral cortex, which contain few or no DA neurons.

The proportion of the [^{3}H]amines in the tissue which was released by amphetamine was a function of the localization of the neurons and not a function of the amount of [^{3}H]amines accumulated by the tissue or of the density of the innervation. For example, the release of [^{3}H]5-HT from the three regions was approximately the same, but the amount accumulated in each region was markedly different. The release of [^{3}H]NE from cerebral cortex was most sensitive to amphetamine, but the amount of [^{3}H]NE accumulated in the cerebral cortex was less than in the other brain regions.

The selectivity of release of the [^{3}H]amines by amphetamine was also examined by observing the accumulation and release of [^{3}H]5-HT and [^{3}H]NE in the presence of five-fold higher concentrations of unlabeled amines. If 5-HT and NE were taken up

and released from NE neurons in the same manner, a higher concentration of unlabeled NE would compete with the labeled [^{3}H]5-HT and, thus, less [^{3}H]5-HT would be available for release by amphetamine. It was observed that there was relatively little interaction between unlabeled NE and the accumulation and release of [^{3}H]5-HT. There was an interaction between unlabeled DA and the release of [^{3}H]5-HT in the absence of amphetamine. This was probably due to displacement of [^{3}H]5-HT from binding sites during the washing phase of the experiments. Amphetamine-induced release of [^{3}H]5-HT was not markedly altered by unlabeled DA. Unlabeled DA had a similar effect on release of [^{3}H]NE from cerebral cortex tissue. However, the effect of DA on the release of the [^{3}H]NE and [^{3}H]5-HT was much less than the effect of the corresponding unlabeled amine.

When the unlabeled amines were added only during the release phase of the experiment, the specificity of release by the various biogenic amines became more obvious. In the case of all three amines, the release of the [^{3}H]amine by its unlabeled analogue occurred with concentrations as low as or lower than the release produced by either amphetamine or the other unlabeled amines. At higher concentrations, DA, NE and amphetamine were all quite effective in releasing [^{3}H]DA from corpus striatum. Each of the four amines had different potencies in releasing [^{3}H]5-HT from corpus striatum. 5-HT was most potent and NE was least potent, with DA and amphetamine of intermediate potency. Similar observations were made with perfused slices of rat corpus striatum.[4] Metaraminol, an analogue of NE, was much more effective in increasing the efflux of [^{3}H]NE than in increasing the efflux of [^{3}H]5-HT.

Although it is difficult to extrapolate from data on release of amines *in vitro* to changes in behavior, there are some obvious consistencies which suggest that the behavioral effects of amphetamine may be related to the selective action on the release of [^{3}H]amines. Taylor and Snyder[26] observed that amphetamine increases spontaneous motor activity in rats with doses about one-fifth of those required to produce a repetitive (stereotyped) gnawing behavior. The majority of the evidence suggests that the increase in spontaneous motor activity is mediated primarily by NE neurons,[2,17,26] while the stereotyped behavior probably involves DA neurons.[17,26–28] These reports are consistent with results from the present study which indicate that the ED_{50} of amphetamine for release of [^{3}H]DA from tissue containing DA neurons is five to ten times that for release of [^{3}H]NE from tissue containing NE neurons. The extrapolation is not entirely clear, however, since there are other reports which suggest that DA neurons may be primarily involved in spontaneous motor activity.[29,30] Another difficulty with extrapolation to the situation in the rat *in vivo* is that, in this species, amphetamine is converted to *p*-hydroxyamphetamine and *p*-hydroxynorephedrine.[31–33] Preliminary evidence from this laboratory[34] suggests that this would not markedly influence the action of amphetamine on NE neurons, since these agents have approximately the same effect on these neurons as does amphetamine. On the other hand, if amphetamine metabolites were released instead of NE, they would probably be less effective than NE in activating the receptor.[35,36]

The role of 5-HT in mediating the behavioral effects of amphetamine is also not entirely clear. Knoll[37] suggested that the psychotomimetic effects obtained with high doses of amphetamine are related to 5-HT metabolism. He observed that the psychotomimetic effects of *p*-bromomethamphetamine are antagonized by pretreatment with *p*-chlorophenylalanine, a substance which depletes 5-HT by inhibition of its synthesis.

This suggests that 5-HT is necessary to obtain the psychotomimetic effects of amphetamine and data from this study indicate that high concentrations of amphetamine release 5-HT from brain tissue containing 5-HT neurons. Fuxe and Ungerstedt[17] also observed that high doses of amphetamine reduced the fluorescence in 5-HT neurons, indicating that amphetamine releases 5-HT from 5-HT neurons. Thus, these studies provide suggestive evidence that amphetamine psychosis may be related to the release of 5-HT by high concentrations of amphetamine.

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