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# COMPARISON OF THE RELEASE OF [$^3$H]DOPAMINE FROM ISOLATED CORPUS STRIATUM BY AMPHETAMINE, FENFLURAMINE AND UNLABELLED DOPAMINE*

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**Abstract**—Amphetamine-induced release of previously accumulated [$^3$H]dopamine ([$^3$H]DA) was compared to the release of [$^3$H]DA produced by unlabelled DA and fenfluramine. Like unlabelled DA, amphetamine was more potent than fenfluramine in releasing [$^3$H]DA in all tissue preparations (untreated, pargyline-treated, and pargyline- and reserpine-treated corpus striatal slices). In tissue treated with both reserpine and pargyline, benztropine greatly reduced the efflux of [$^3$H]DA produced by amphetamine and unlabelled DA but had only a slight effect on fenfluramine-induced release of [$^3$H]DA. In the same tissue preparation, $Q_{10}$ values for the release of [$^3$H]DA produced by $3 \times 10^{-7}$ M amphetamine (1.8) and $3 \times 10^{-6}$ M unlabelled DA (1.7) were similar to that for the spontaneous release of [$^3$H]DA (1.7). However, when the concentrations of amphetamine and unlabelled DA were increased to $10^{-4}$ M, the $Q_{10}$ values for the release of [$^3$H]DA were diminished at the lower temperatures. These results suggest that amphetamine may release [$^3$H]DA by two mechanisms: (1) by accelerated exchange diffusion due to its use of the DA uptake carrier to enter into neurons (this would predominate at low concentrations of amphetamine), and (2) by passive entrance into neurons and displacement of [$^3$H]DA from binding sites (this would predominate at high concentrations of amphetamine).

Evidence suggests that the release of norepinephrine from central nerve endings by low concentrations of amphetamine requires the uptake of amphetamine by the neuronal uptake system [1–3]. It has been further suggested [3, 4] that, once amphetamine is inside the neuron and displaces norepinephrine from intraneuronal binding sites, the displaced amine exits the nerve ending by using the neuronal uptake system so that it is operating in the reverse direction. Less information is available concerning the mechanism of amphetamine-induced release of dopamine (DA) from corpus striatum but similar mechanisms are thought to be involved when low concentrations of amphetamine are investigated [5, 6]. With higher concentrations of amphetamine, the binding sites in the neuronal uptake system are saturated and amphetamine probably enters primarily by passive diffusion [1]. The current studies were designed to contrast the effect of amphetamine on the release of [$^3$H]DA with that of fenfluramine, which is thought to enter the nerve ending by passive diffusion at all concentrations [7], and of unlabelled DA, which is thought to enter by the neuronal uptake system at all concentrations [8, 9]. In order to eliminate the confounding variable of vesicular storage of DA and to optimize the movement of the transport system in the efflux direction, the cytoplasmic DA concentration can be increased by labelling slices of corpus striatum with [$^3$H]DA in the presence of pargyline and reserpine. Pargyline inhibits monoamine oxidase, and reserpine inhibits vesicular storage of DA. Under these conditions, accumulation of cytoplasmic [$^3$H]DA is greatly enhanced [10, 11].

If amphetamine-induced release of [$^3$H]DA is primarily dependent upon its accumulation in the nerve endings by a carrier-mediated transport process, then a drug such as benztropine [12] which can block the DA uptake carrier system would inhibit the uptake of amphetamine and thereby reduce amphetamine-induced release of [$^3$H]DA from nerve endings. Therefore, the effect of benztropine on the efflux of [$^3$H]DA produced by amphetamine, unlabelled DA and fenfluramine was investigated.

The uptake of [$^3$H]DA into corpus striatal synaptosomes has been shown to be temperature dependent [13] with a $Q_{10}$ of approximately 1.7 between 29° and 37°. This $Q_{10}$ indicates that the uptake of DA is mediated by a facilitated diffusion system since the value approximates 2.0 [14]. If release occurs primarily by reversal of the neuronal uptake system, the $Q_{10}$ for release should also approximate 2.0. Thus, an Arrhenius analysis may help to distinguish release by those drugs which utilize the facilitated diffusion system from those drugs which enter by passive diffusion.

## MATERIALS AND METHODS

*Drug-induced release of* [$^3$H]*DA from reserpine and pargyline-treated corpus striatum.* Male Sprague–Dawley rats weighing 200–250 g were pretreated with reserpine (2.5 mg/kg, s.c., 16–18 hr) and decapitated, and their brains were removed. The

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corpus striatum was dissected and cut into two pieces, and the wet weight was determined (about 10–12 mg each). The tissue pieces were placed in chromium wire baskets and incubated at 37° in Krebs–Henseleit buffer [15] containing $10^{-4}$ M pargyline for 10 min, washed, and then incubated with $5 \times 10^{-7}$ M [$^3$H]DA and $10^{-4}$ M β-thujaplicin (a catechol-*O*-methyltransferase inhibitor) for 20 min. At the end of the incubation, tissue samples were transferred to tubes containing 2 ml of tracer-free medium. Efflux of [$^3$H]DA was followed for 60 min. The tissue was inserted into fresh medium every 5 min, and the drugs were present from 30 to 60 min during efflux. At the end of the incubation, the tissue was homogenized in 2.5 ml of 2 N HCl. Radioactivities in both medium and tissue were determined by liquid scintillation spectrometry. The result was expressed as a rate coefficient ($f$) in $\text{min}^{-1}$, as described by Paton [4], and was calculated as follows:

$$f = \frac{\Delta A}{\Delta t \cdot At}$$

where $At$ is the [$^3$H]DA content of the tissue at various times during efflux and was calculated by adding successively in reverse order the amount of [$^3$H]DA released into each tube during the efflux periods to that remaining in the tissue at the end of each experiment. $\Delta A$ represents the [$^3$H]DA lost in the time interval $\Delta t$.

In the experiment in which efflux was expressed as percent release, a slightly modified procedure was used. After labelling with [$^3$H]DA, the tissue samples were transferred to a series of tubes to wash nonspecifically bound [$^3$H]DA from the tissue. The washing procedure was over a 30-min period as described above. The tissue samples were then transferred to tubes containing 2 ml of drugs to be tested. At the end of a 20-min incubation, the tissue was homogenized in 2.5 ml of 2 N HCl. Radioactivities in both medium and tissue were determined as indicated above. Percent release was calculated as follows:

$$\frac{\text{dpm in medium}}{\text{dpm in medium + dpm in tissue}} \times 100$$

*Drug-induced release of* [$^3$H]*DA from normal corpus striatum.* When the tissue was not exposed to pargyline, it was necessary to separate [$^3$H]DA from $^3$H-deaminated metabolites. Therefore, at the end of the 20-min incubation, the medium was immediately acidified with 2 ml of 2 N HCl and the tissue was homogenized with 2.5 ml of 2 N HCl. The radioactivity was extracted from the precipitated protein by centrifugation (10,000 *g* for 10 min). Unlabelled DA (100 μg) in 15% EDTA was added to both medium and tissue extracts prior to homogenization. DA was added as a carrier substance and for the determination of recoveries. The [$^3$H]DA in the medium and tissue extracts was separated from their $^3$H-deaminated metabolites by cation exchange chromatography on Dowex 50 $Na^+$ [9, 16]. The radioactivity in each fraction was determined by liquid scintillation spectrometry. The total amine in the eluate was corrected for recovery which was determined by spectrofluorometric measurement. The results were expressed as the percentage of [$^3$H]DA in the incubation medium calculated as:

$$\frac{\text{[}^3\text{H]DA in medium}}{\text{[}^3\text{H]DA in medium + [}^3\text{H]DA in tissue}} \times 100$$

*Inhibition by benztropine of* [$^3$H]*DA uptake into chopped corpus striatum.* The methods of Ziance and Rutledge [6] were used. Male Sprague–Dawley rats (200–250 g) were decapitated, and the brain was removed. The corpus striatum was dissected and chopped into fine pieces with a McIlwain tissue chopper. The chopped tissue was suspended in a physiological salt solution, and aliquots of the tissue suspension were added to tubes containing various concentrations of benztropine. The tissue mixture was preincubated at 37° for 10 min, after which [$^3$H]DA (sp. act. 5.0 Ci/mmole) was added to the mixture to obtain an incubation volume of 2.0 ml and a final concentration of $5 \times 10^{-7}$ M [$^3$H]DA, and the incubation was continued for an additional 20 min. The uptake of [$^3$H]DA into the tissue was terminated by immediate centrifugation, 4°. The supernatant fraction was decanted and the tissue was washed with physiological salt solution (4°), and the wash was combined with the supernatant fraction. Ethanol was added to the tissue pellet and the tissue was homogenized using a glass homogenizer. The [$^3$H]DA in the tissue extract and medium fraction was determined by liquid scintillation spectrometry and the uptake was expressed as the tissue-to-medium ratio (T/M) of the radioactivity as follows:

$$\frac{\text{dpm in the tissue/g wet wt tissue}}{\text{dpm in the medium/ml medium}}$$

The percent inhibition was determined by the following formula:

$$\frac{(R_c - R_i)}{(R_c - R_o)} \times 100$$

where $R_c$ is the T/M ratio for the control sample incubated in the absence of benztropine. $R_i$ is the T/M ratio for the sample incubated in the presence of benztropine, and $R_o$ is the T/M ratio for the sample incubated in the presence of a maximal concentration of benztropine ($10^{-3}$ M).

*Release of* [$^3$H]*DA from chopped corpus striatum by benztropine.* The method of Ziance and Rutledge [16] was used. Chopped corpus striatum was prepared as described for the uptake experiments. The tissue was preincubated with [$^3$H]DA and then washed several times to remove nonspecifically bound [$^3$H]DA. After washing, the tissue was incubated with various concentrations of benztropine at 37° for 30 min. At the end of the incubation, the medium was separated from the tissue by centrifugation, the tissue was washed with ice-cold physiological salt solution, and the wash was combined with the medium. The [$^3$H]DA in the medium and tissue was then separated from the [$^3$H]deaminated metabolites as previously described. Release of [$^3$H]DA from the tissue by benztropine was expressed as [$^3$H]DA in the medium as a percentage of the total [$^3$H]DA in the tissue and medium.

*Arrhenius analysis of drug-induced release of* [$^3$H]*DA from pargyline- and reserpine-treated corpus*

*striatal slices.* The procedures were identical to those described for drug-induced release of [$^3$H]DA In reserpine- and pargyline-treated corpus striatum except that the release was measured at various temperatures. For each temperature, the tissue was preincubated with [$^3$H]DA at 37°, washed at 37° for 20 min, and then incubated at the test temperature for 10 min. After washing, the tissue was incubated in the presence or absence of drugs at the same test temperature for 5 min. The release of [$^3$H]DA into medium was expressed as a release ratio, calculated as dpm in medium/(dpm in medium plus dpm in tissue). Arrhenius analysis was obtained by plotting the logarithm of the rate of release which was expressed as the (release ratio × 100)/5 min versus the reciprocal of the absolute temperature. The plot was determined by linear regression analysis, and the $E_a$ and the $Q_{10}$ for the rate-limiting step were calculated from the slope of the plot as described in Table 2.

*Substances.* *d*-Amphetamine sulfate was obtained from the Smith, Kline & French Laboratories (Philadelphia, PA), and fenfluramine HCl from the A. H. Robins Co. (Richmond, VA). [$^3$H]Dopamine (5 Ci/mmole) was purchased from the Amersham/Searle Corp. (Arlington Heights, IL). 3-Hydroxytyramine (DA) HCl and pargyline HCl were obtained from the Sigma Chemical Co. (St. Louis, MO). β-Thujaplicin was obtained from the Aldrich Chemical Co. (Milwaukee, WI), and reserpine (Serapsil) from the Ciba-Geigy Corp. (Summit, NJ). Benztropine mesylate was donated by Merck & Co., Inc. (Rahway, NJ).

## RESULTS

*Effects of various drugs on the efflux of* [$^3$H]*DA from corpus striatum.* In Fig. 1, the efflux of [$^3$H]DA from pargyline- and reserpine-treated corpus striatum proceeded exponentially with an initially rapid rate, followed by a slower rate which, after about 30 min, approximated a straight line. This is consistent with the hypothesis that efflux was occurring predominantly from a single compartment after a 30-min incubation. Addition of $10^{-5}$ M amphetamine or $10^{-5}$ M unlabelled DA markedly increased the release of [$^3$H]DA, and the maximum rate of efflux produced by the two drugs occurred within 5 min after drug administration. Fenfluramine ($10^{-4}$ M) also produced an increase in the efflux of [$^3$H]DA, producing its maximum stimulation of efflux at about 10 min after adding the drug.

Amphetamine, unlabelled DA and fenfluramine all produced dose-related increases in the release of [$^3$H]DA (Figs. 2–4). In all three tissue preparations being studied, amphetamine and unlabelled DA were much more potent than fenfluramine in releasing [$^3$H]DA. The $EC_{50}$ values (μM) of the three drugs in the various tissue preparations were as follows: untreated tissue (Fig. 2) (amphetamine, 4.4 ± 1.9; unlabelled DA, 11.5 ± 3.3; fenfluramine, 260.0 ± 68.0); pargyline-treated tissue (Fig. 3) (amphetamine, 4.0 ± 2.0; unlabelled DA, 13.6 ± 5.8; fenfluramine, 167 ± 1.0); pargyline- and reserpine-treated tissue (Fig. 4) (amphetamine, 0.8 ± 0.1; unlabelled DA, 7.5 ± 1.4; fenfluramine, 183.0 ± 13.0).

In untreated tissue, amphetamine-induced release of [$^3$H]DA into the medium was correlated with a decrease of [$^3$H]DA in the tissue. In addition, the amount of the $^3$H-deaminated metabolites released into the medium was either decreased, or exhibited no change, compared to control. This latter phenomenon was not observed with unlabelled DA and

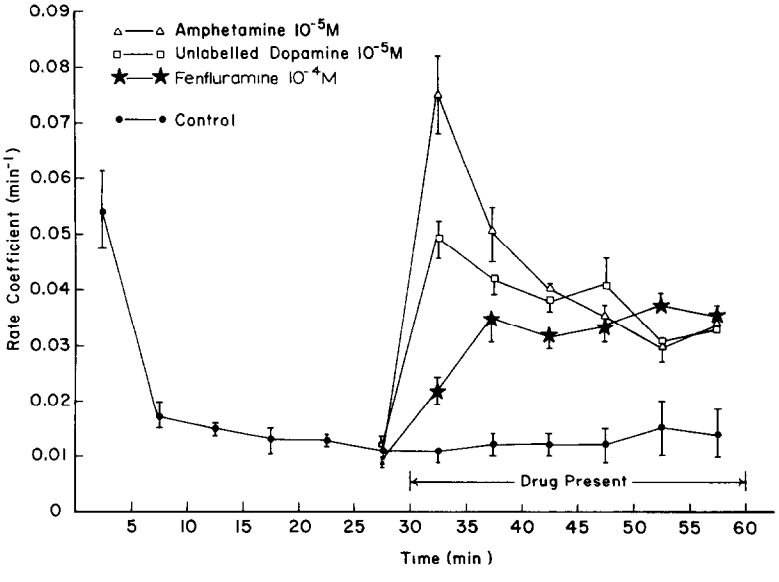


Fig. 1. Time course of spontaneous release of [$^3$H]DA by $10^{-5}$ M amphetamine, $10^{-5}$ M unlabelled DA and $10^{-4}$ M fenfluramine from pargyline- and reserpine-treated corpus striatal slices. Nerve endings were labelled by incubation of the tissue with [$^3$H]DA. The tissue was then transferred every 5 min to a series of tubes containing tracer-free physiological medium. Release of [$^3$H]DA into the medium was expressed as a rate coefficient (*f*) in $min^{-1}$ as described in Materials and Methods. Each value is the mean ± S.E.M. of three to four determinations.

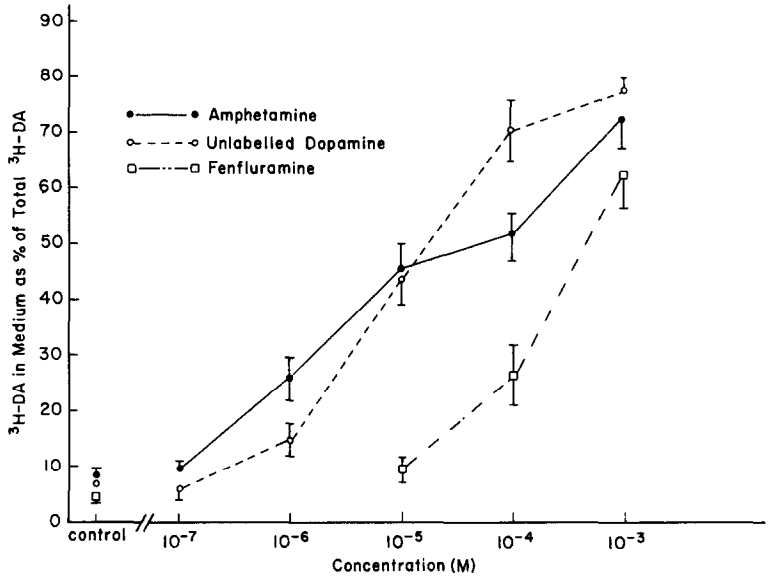


Fig. 2. Release of [$^3$H]DA from untreated corpus striatal slices produced by amphetamine, unlabelled DA and fenfluramine. Neurons were labelled by incubation of tissue with [$^3$H]DA. The tissue was washed and incubated for 20 min in the presence or absence of various drugs. The deaminated metabolites were separated from the amines by chromatography on a Dowex-50 $Na^+$ column. Release was expressed as [$^3$H]DA in the medium as percentage of total [$^3$H]DA in the medium and tissue. Each value is the mean ± S.E.M. of three to five determinations.

fenfluramine. At those concentrations that released [$^3$H]DA into the medium (except $10^{-3}$ M unlabelled DA) unlabelled DA and fenfluramine significantly increased the deaminated metabolites of [$^3$H]DA (Table 1, Fig. 5).

*Effect of benztropine on the uptake and spontaneous release of* [$^3$H]*DA and on drug-induced efflux of* [$^3$H]*DA.* The effects of benztropine on release and uptake of [$^3$H]DA were studied in separate experiments using untreated, chopped corpus stria-

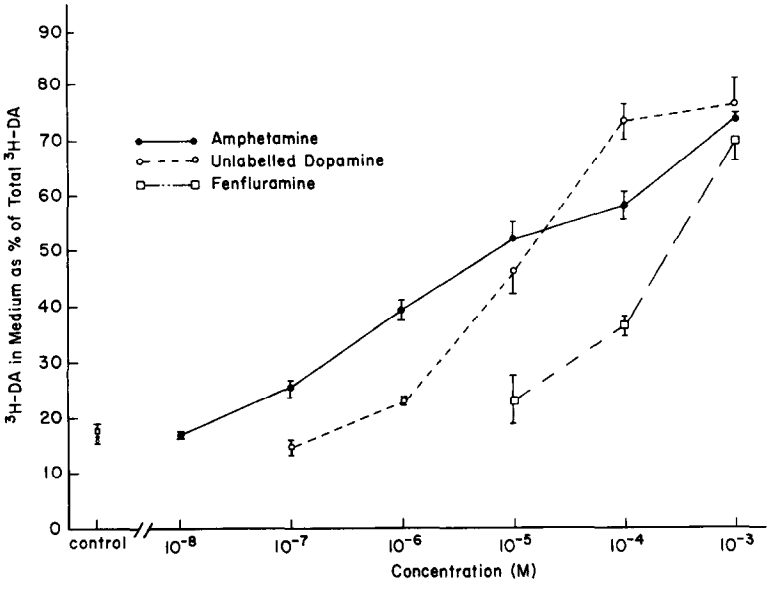


Fig. 3. Release of [$^3$H]DA from pargyline-treated corpus striatal slices produced by amphetamine, unlabelled DA and fenfluramine. Neurons were labelled by incubation of the tissue with [$^3$H]DA. The tissue was washed and incubated for 20 min in the presence or absence of various drugs. Release was expressed as [$^3$H]DA in the medium as percentage of total [$^3$H]DA in the medium and tissue. Each value is the mean ± S.E.M. of three to five determinations.

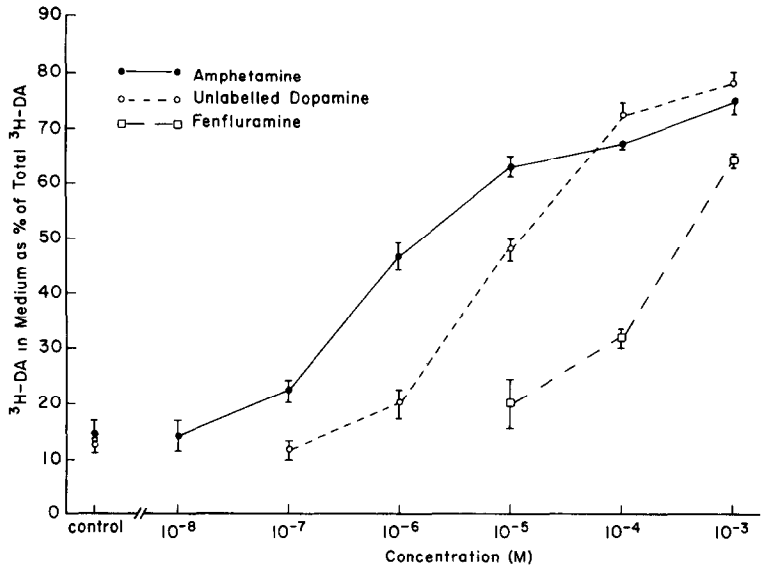


Fig. 4. Release of [$^3$H]DA from pargyline- and reserpine-treated corpus striatal slices produced by amphetamine, unlabelled DA and fenfluramine. Neurons were labelled by incubation of the tissue with [$^3$H]DA. The tissue was washed and incubated for 20 min in the presence or absence of various drugs. Release was expressed as [$^3$H]DA in the medium as percentage of total [$^3$H]DA in medium and tissue. Each value is the mean ± S.E.M. of three to five determinations.

tum (Fig. 6). Benztropine produced a concentration-related effect on both release of [$^3$H]DA and inhibition of the uptake of [$^3$H]DA into the tissue. Benztropine was more potent in inhibiting the uptake of [$^3$H]DA than in releasing [$^3$H]DA. The concentrations required to produce a half-maximum response were 4.8 ± 1.0 $\mu$M for uptake inhibition and 52.1 ± 5.9 $\mu$M for release. In the same tissue preparations, $10^{-5}$ M benztropine greatly inhibited the uptake of [$^3$H]DA (by 61%), whereas it caused only a slight release of [$^3$H]DA (about 10%). When $10^{-5}$ M benztropine was incubated with pargyline- and reserpine-treated tissue in the presence of various releasing drugs, it was observed that the efflux of [$^3$H]DA produced by amphetamine was reduced significantly (Fig. 7). A decrease in the efflux of [$^3$H]DA was also observed with unlabelled DA in the presence of benztropine (Fig. 8). However, the

Table 1. Effect of amphetamine, unlabelled DA and fenfluramine on the release of [$^3$H]DA from untreated corpus striatal slices*

| Concn (M) | Drugs | 3-Methoxytyramine + DA (M) | 3-Methoxytyramine + DA (T) | Total deaminated catabolites (M) | Total deaminated catabolites (T) |
|---|---|---|---|---|---|
| | Control | 5.3 ± 0.6 | 58.5 ± 3.9 | 17.8 ± 1.1 | 18.0 ± 3.0 |
| $10^{-6}$ | Amphetamine | 9.7 ± 1.5† | 52.7 ± 2.5† | 14.5 ± 0.7† | 12.3 ± 0.7 |
| | Unlabelled DA | 9.4 ± 2.6 | 53.4 ± 4.1 | 22.3 ± 1.1† | 13.7 ± 2.7 |
| $10^{-5}$ | Amphetamine | 33.2 ± 2.5† | 40.7 ± 3.6† | 14.4 ± 0.8† | 11.7 ± 1.3† |
| | Unlabelled DA | 26.1 ± 3.4† | 34.3 ± 3.9† | 24.3 ± 1.6† | 16.2 ± 1.1 |
| | Fenfluramine | 6.5 ± 1.3 | 61.2 ± 0.9 | 17.8 ± 0.9 | 14.6 ± 0.4 |
| $10^{-4}$ | Amphetamine | 44.4 ± 2.0† | 33.5 ± 3.9† | 12.3 ± 0.7† | 9.4 ± 1.6† |
| | Unlabelled DA | 47.4 ± 3.0† | 18.1 ± 0.7† | 23.2 ± 2.0† | 11.3 ± 0.9† |
| | Fenfluramine | 15.5 ± 2.5† | 44.4 ± 3.7† | 25.4 ± 1.3† | 14.3 ± 0.3† |
| $10^{-3}$ | Amphetamine | 61.4 ± 4.8† | 18.7 ± 3.1† | 10.7 ± 0.4† | 9.0 ± 1.4† |
| | Unlabelled DA | 62.5 ± 1.6† | 18.2 ± 1.8† | 11.9 ± 0.9† | 7.3 ± 0.8† |
| | Fenfluramine | 39.2 ± 4.3† | 23.6 ± 3.2† | 25.7 ± 0.7† | 11.5 ± 2.1† |

* Neurons were labelled by incubation of the untreated corpus striatal slices with [$^3$H]DA. The tissue was washed and incubated for 20 min with test drug. The tritiated material from media and tissue samples was passed over Dowex-50 $Na^+$ columns. Both the effluent (total deaminated catabolites) and eluate (DA and 3-methoxytyramine) were collected. Values are expressed as percentages of the total tritiated material present in each fraction. Each value is the mean ± S.E.M. of three to five determinations. Student's *t*-test was performed for the statistical analysis. M = in medium; T = in tissue.

† $P < 0.05$ (different from control).

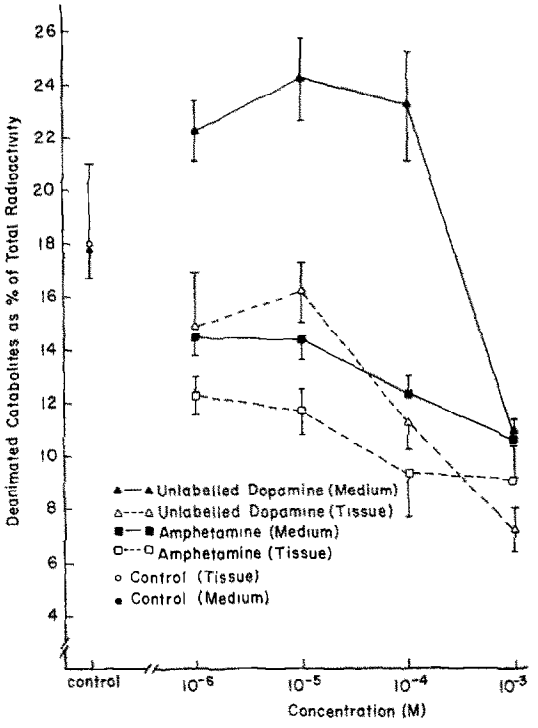


Fig. 5. Formation of deaminated metabolites from [$^3$H]DA, induced by amphetamine and unlabelled DA, in untreated corpus striatal slices. Neurons were labelled by incubation of the tissue with [$^3$H]DA. The tissue was washed and incubated for 20 min in the presence or absence of amphetamine or unlabelled DA. The deaminated metabolites were separated from the amines by cation-exchange chromatography using Dowex-50 $Na^+$ columns. $^3$H-Deaminated metabolites, formed in the medium and tissue, were expressed as percentage of the total tritium initially accumulated by corpus striatal neurons. Each value is the mean ± S.E.M. of three to five determinations.

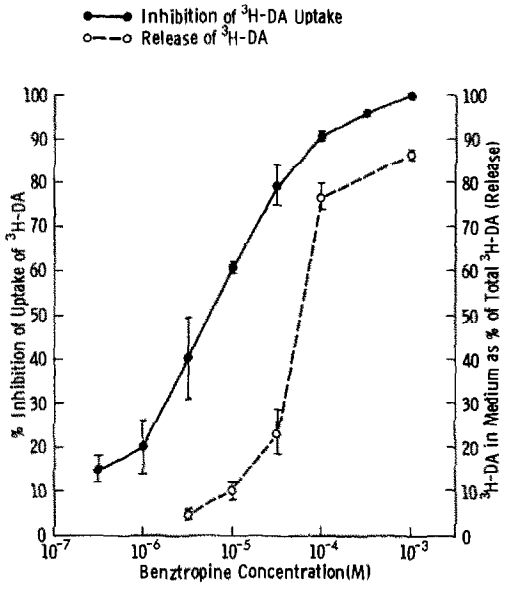


Fig. 6. Effect of benztropine on neuronal uptake and release of [$^3$H]DA in chopped corpus striatum. Inhibition of neuronal uptake (left ordinate) was expressed as a percentage of control values. Release of [$^3$H]DA (right ordinate) was expressed as [$^3$H]DA in the medium as percentage of total [$^3$H]DA in medium and tissue. Control release of [$^3$H]DA into the medium was 4.1 ± 0.7% and was subtracted from each of the experimental values. The mean tissue/medium ratio for uptake in control samples was 46.4 ± 4.4. Each value is the mean ± S.E.M. of four determinations.

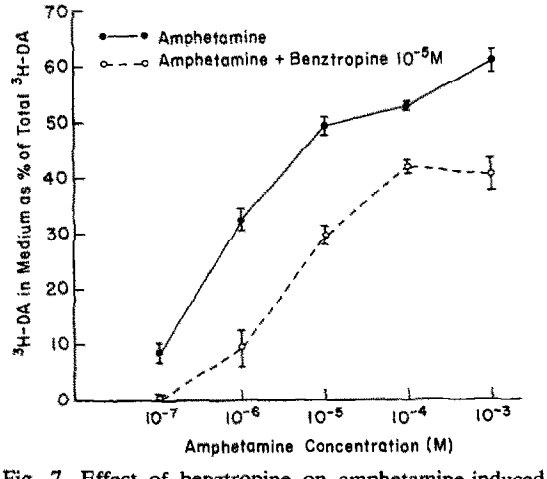


Fig. 7. Effect of benztropine on amphetamine-induced release of [$^3$H]DA in pargyline- and reserpine-treated corpus striatal slices. Neurons were labelled by incubation of the tissue with [$^3$H]DA. The tissue was washed and incubated for 20 min with amphetamine or amphetamine plus $10^{-5}$ M benztropine. Release was expressed as [$^3$H]DA in the medium as percentage of total [$^3$H]DA in medium and tissue. Basal release was subtracted from each of the amphetamine values and was 13.0 ± 0.9% for control and 21.1 ± 0.3% for $10^{-5}$ M benztropine. Each value is the mean ± S.E.M. of three to five determinations.

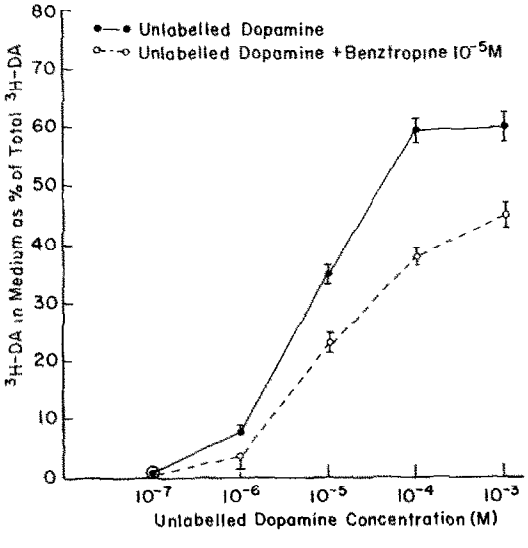


Fig. 8. Effect of benztropine on unlabelled DA-induced release of [$^3$H]DA in pargyline- and reserpine-treated corpus striatal slices. Neurons were labelled by incubation of the tissue with [$^3$H]DA. The tissue was washed and incubated for 20 min with unlabelled DA or unlabelled DA plus $10^{-5}$ M benztropine. Release was expressed as [$^3$H]DA in the medium as percentage of total [$^3$H]DA in medium and tissue. Basal release was subtracted from each of the unlabelled DA values and was 13.0 ± 0.9% for control and 21.2 ± 0.3% for $10^{-5}$ M benztropine. Each value is the mean ± S.E.M. of three to five determinations.

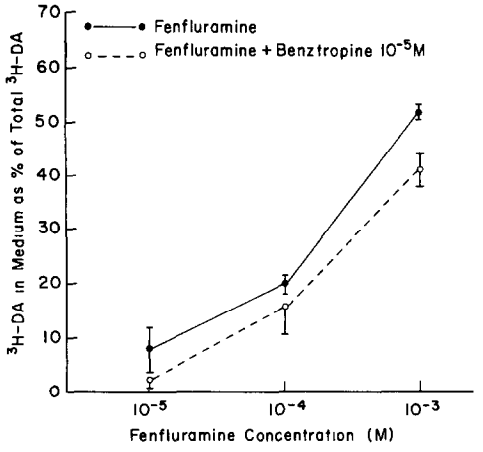


Fig. 9. Effect of benztropine on fenfluramine-induced release of [$^3$H]DA in pargyline- and reserpine-treated corpus striatal slices. Neurons were labelled by incubation of the tissue with [$^3$H]DA. The tissue was washed and incubated for 20 min with fenfluramine or fenfluramine plus $10^{-5}$ M benztropine. Release was expressed as [$^3$H]DA in the medium as percentage of total [$^3$H]DA in the medium and tissue. Basal release was subtracted from each of the fenfluramine values and was 13.0 ± 0.9% for control and 21.2 ± 0.3% for $10^{-5}$ M benztropine. Each value is the mean ± S.E.M. of three to five determinations.

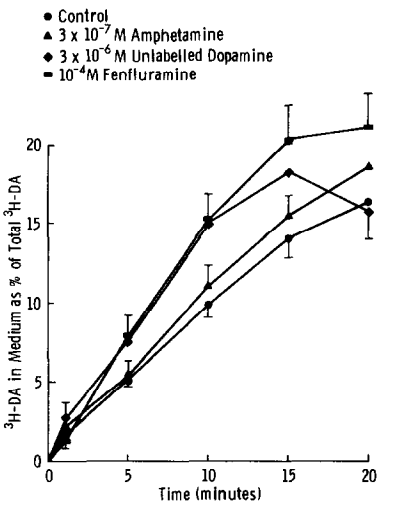


Fig. 10. Time courses for spontaneous release and the release of [$^3$H]DA produced by 3 × $10^{-7}$ M amphetamine, 3 × $10^{-6}$ M unlabelled DA and $10^{-4}$ M fenfluramine in pargyline- and reserpine-treated corpus striatum. Nerve endings were labelled by incubation of the tissue with [$^3$H]DA. The tissue was washed in tracer-free medium at 37° for 30 min and then incubated in the presence or absence of various drugs. Incubation was terminated at various times between 2 and 20 min. Release of [$^3$H]DA into the medium was expressed as [$^3$H]DA in the medium as percentage of the total [$^3$H]DA in medium and tissue. The drug-induced release occurring at each concentration of drug was corrected for control release. Each value is the mean ± S.E.M. of three to five determinations.

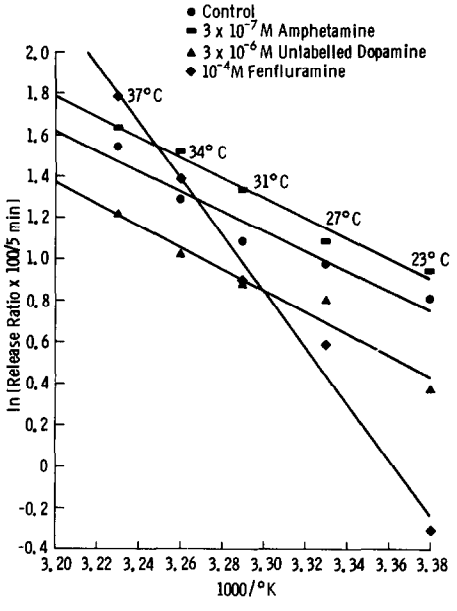


Fig. 11. Arrhenius plot of spontaneous release and the release of [$^3$H]DA produced by 3 × $10^{-7}$ M amphetamine, 3 × $10^{-6}$ M unlabelled DA and $10^{-4}$ M fenfluramine in pargyline- and reserpine-treated corpus striatal slices. Nerve endings were labelled by incubation of tissue with [$^3$H]DA. The tissue was washed in tracer-free medium at 37° for 20 min and then at the test temperature for 10 min. The washed tissue was incubated in the presence or absence of test drugs at each temperature for 5 min. Release was expressed as the ratio of [$^3$H]DA in the medium to [$^3$H]DA in the medium plus [$^3$H]DA in the tissue. Control release ratio was subtracted from the drug-induced release ratio at each given temperature. Each value is the mean of four to seven determiantions.

efflux of [$^3$H]DA produced by fenfluramine was not affected by benztropine until $10^{-3}$ M fenfluramine (Fig. 9). At $10^{-3}$ M fenfluramine, benztropine slightly reduced the fenfluramine-induced release of [$^3$H]DA.

*Arrhenius analysis of drug-induced release of* [$^3$H]*DA*. In Fig. 10, the spontaneous release of [$^3$H]DA, and the release of [$^3$H]DA produced by amphetamine (3 × $10^{-7}$ M), unlabelled DA (3 × $10^{-6}$ M) and fenfluramine ($10^{-4}$ M) in pargyline- and reserpine-treated corpus striatum, were linear with the duration of incubation for the first 10 min, and release reached plateau after 15 min. Therefore, 5 min of incubation time when release was still linear was selected for the Arrhenius analysis.

In Fig. 11, a comparison of Arrhenius plots of spontaneous release and release of [$^3$H]DA produced by amphetamine (3 × $10^{-7}$ M), unlabelled DA (3 × $10^{-6}$ M) and fenfluramine ($10^{-4}$ M) was made. The concentration selected for each drug was based on the minimally effective concentration needed to produce release of [$^3$H]DA during a 5-min incubation. In contrast to fenfluramine, the slopes of the Arrhenius plots for the efflux of both [$^3$H]DA produced by amphetamine and unlabelled DA were similar to that observed for spontaneous release of [$^3$H]DA. The respective $E_a$ (kcal/mole) and $Q_{10}$ values derived from the slopes of the Arrhenius plots are as follows (Table 2): spontaneous release of [$^3$H]DA (9.5, 1.7); 3 × $10^{-7}$ M amphetamine (9.6, 1.7); 3 × $10^{-6}$ M unlabelled DA (10.4, 1.8); and

Table 2. Effect of temperature on the release of [$^3$H]DA produced by various drugs*

| Drugs | $E_a$ (kcal/mole) | $Q_{10}$ |
|---|---|---|
| Control | 9.45 (37°–23°) | 1.7 |
| Amphetamine ($3 \times 10^{-7}$ M) | 9.64 (37°–23°) | 1.7 |
| Unlabelled DA ($3 \times 10^{-6}$ M) | 10.40 (37°–23°) | 1.8 |
| Fenfluramine ($10^{-4}$ M) | 26.73 (37°–23°) | 4.3 |
| Amphetamine ($10^{-4}$ M) | 11.23 (37°–30°) | 1.7 |
| | 1.98 (30°–23°) | 1.1 |
| Unlabelled DA ($10^{-4}$ M) | 10.97 (37°–29°) | 1.7 |
| | 6.12 (29°–23°) | 1.3 |

$E_a$ and $Q_{10}$ are derived from the data presented in Figs. 10 and 11 by the following equations:
$E_a = R \times$ slope where $R = 1.98$ cal°K$^{-1}$mole$^{-1}$.
$Q_{10} = e^{10E_a/RT_1T_2}$ where $R = 1.98$ cal°K$^{-1}$mole$^{-1}$.
$T_1 = 37°$.
$T_2 = 27°$.

$10^{-4}$ M fenfluramine (26.7, 4.3). When the concentrations of amphetamine and unlabelled DA were increased to $10^{-4}$ M, both drugs produced broken Arrhenius plots (Fig. 12). At higher temperatures (29° to 37°), the slopes of Arrhenius plots produced by amphetamine and unlabelled DA were still similar to that of spontaneous release of [$^3$H]DA. However, when the incubation temperature was decreased (30° to 23°), the slopes of the Arrhenius plots of the release produced by amphetamine and unlabelled DA were also reduced. In addition, the degree of reduction in the slopes was more pronounced in amphetamine-induced release compared to that of unlabelled DA. The respective $E_a$ (kcal/mole) and $Q_{10}$ values derived from the slopes of the Arrhenius plots are as follows (Table 2): $10^{-4}$ M amphetamine, 11.23, 1.7 (between 37° and 30°), and 1.98, 1.1 (between 30° and 23°); $10^{-4}$ M unlabelled DA, 10.97, 1.7 (between 37° and 29°) and 6.12, 1.3 (between 29° and 23°).

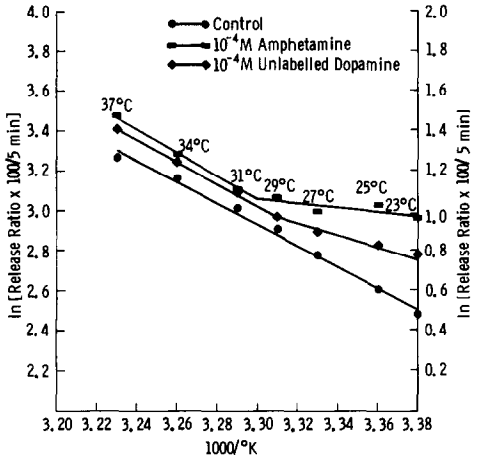


Fig. 12. Arrhenius plot of spontaneous release and the release of [$^3$H]DA by $10^{-4}$ M amphetamine and $10^{-4}$ M unlabelled DA in pargyline- and reserpine-treated corpus striatal slices. Nerve endings were labelled with [$^3$H]DA by incubation of the tissue with the tracer at 37° for 20 min. The tissue was washed in tracer-free medium for 30 min. The washed tissue was incubated in the presence or absence of test drugs at each test temperature for 5 min. Release was expressed as the ratio of [$^3$H]DA in the medium to [$^3$H]DA in the medium plus [$^3$H]DA in the tissue (left ordinate: drug-induced release; right ordinate: control release). Control release was subtracted from the drug-induced release at each temperature. Each value is the mean of four to seven determinations.

## DISCUSSION

It was observed in this study that benztropine inhibits amphetamine-induced release of [$^3$H]DA. This is consistent with the results obtained by Raiteri *et al.* [6] using nomifensine which, like benztropine, blocks DA uptake. These findings, together with the observation that benztropine inhibits both amphetamine- and unlabelled DA-induced release of [$^3$H]DA while having little effect on fenfluramine-induced release of [$^3$H]DA, suggest that the transport of amphetamine and unlabelled DA by a carrier-mediated process is necessary for the release of [$^3$H]DA produced by the two drugs. On the other hand, fenfluramine-induced release of [$^3$H]DA is not dependent on the carrier-mediated transport of the drug. It would appear that a drug which blocks the carrier system would be more potent in reducing the efflux of [$^3$H]DA produced by amphetamine and unlabelled DA than in blocking the release of [$^3$H]DA produced by fenfluramine. In the former case, the inhibition of the uptake of the releasing drug (amphetamine or unlabelled DA) would occur at sites on the outside of the neuronal membrane. Benztropine would limit access of the releasing drug to the neuron, thus decreasing the observed release of [$^3$H]DA. However, in the latter case, the inhibition of fenfluramine-induced release of [$^3$H]DA would not occur until after fenfluramine had entered the nerve ending and displaced [$^3$H]DA from intraneuronal binding sites.

In contrast to amphetamine, an increase in the deaminated metabolites of [$^3$H]DA was observed with both fenfluramine and unlabelled DA. This is consistent with previous experiments which had demonstrated that amphetamine-induced release of [$^3$H]DA is associated with a decrease in deaminated metabolites while fenfluramine-induced release is associated with an increase in deaminated metabolites [7, 16]. It was suggested that fenfluramine enters the neuron by passive diffusion and is thereby unable to provide carriers inside the membrane to allow for rapid efflux of the displaced [$^3$H]DA. Therefore, the access of displaced [$^3$H]DA to monoamine oxidase is increased. The increase in [$^3$H]-deaminated metabolites associated with unlabelled DA-induced release of [$^3$H]DA may be due to enhanced displacement of [$^3$H]DA from vesicular binding sites so that the binding sites on the carrier available on the inside of the membrane are saturated. Thus, the displaced [$^3$H]DA has increased access to intraneuronal monoamine oxidase, and the excess displaced [$^3$H]DA is metabolized by intraneuronal monoamine oxidase. It is possible that dif-

ferences in the formation of deaminated metabolites might be due to inhibition of monoamine oxidase by amphetamine. This effect is most pronounced at high amphetamine concentrations [17].

The $Q_{10}$ for a kinetic study generally represents the physical properties of the rate-limiting step involved in the process. We have demonstrated that the $Q_{10}$ for the spontaneous release of [$^3$H]DA from corpus striatal tissue slices treated with pargyline and reserpine is 1.7, which is the same as that for the uptake of [$^3$H]DA into corpus striatal synaptosomes observed by Holz and Coyle [13]. Both $Q_{10}$ values are close to 2, suggesting that both processes may be carrier-mediated, and it is consistent with the hypothesis that one process is the reverse of the other. It is noteworthy that the $Q_{10}$ for the release of [$^3$H]DA produced by low concentrations of amphetamine and unlabelled DA is similar to that for the spontaneous release of [$^3$H]DA. This indicates that the rate-limiting step for the former two processes may also involve a facilitated diffusion system. This would promote the entry of amphetamine or unlabelled DA into the nerve ending or the diffusion of [$^3$H]DA out of the nerve ending, or both. On the other hand, the $Q_{10}$ for fenfluramine-induced release is much greater. This indicates that the rate-limiting step for fenfluramine-induced release of [$^3$H]DA involves a separate process which requires a higher energy of activation. The mechanism responsible for the higher $Q_{10}$ observed for fenfluramine-induced release is not known.

A high concentration ($10^{-4}$ M) of fenfluramine was selected to obtain an Arrhenius plot since fenfluramine is not a potent releasing agent. To ensure that the greater $Q_{10}$ obtained with fenfluramine-induced release of [$^3$H]DA was not the result of a nonspecific membrane-stabilizing effect caused by a high concentration of the drug, we further examined the Arrhenius data of [$^3$H]DA efflux produced by high concentrations ($10^{-4}$ M) of unlabelled DA and amphetamine in pargyline- and reserpine-treated corpus striatal slices. At high concentrations, the Arrhenius plots obtained for both amphetamine and unlabelled DA were discontinuous. The mechanism by which amphetamine enters into the nerve ending has been debated for many years. It was first suggested that amphetamine enters nerve endings by passive diffusion since it is relatively lipophilic and can penetrate lipid membranes [18–20]. However, indirect evidence suggests that amphetamine can also enter the neuron by the neuronal uptake system utilized by catecholamines [1, 6]. Based on the results obtained in the present study, it is likely that amphetamine may release [$^3$H]DA by two simultaneous mechanisms as proposed in Fig. 13. These two mechanisms include: (1) accelerated exchange diffusion, and (2) passive diffusion into the neuron followed by direct displacement of [$^3$H]DA from binding sites. The rate of [$^3$H]DA release produced by amphetamine which enters into neurons by the carrier system would be faster than the rate produced by amphetamine which has entered passively, and the overall release observed would be a summation of rates of two individual processes. At low concentrations of amphetamine, the carriers are probably not saturated by amphetamine, and it is likely that the release

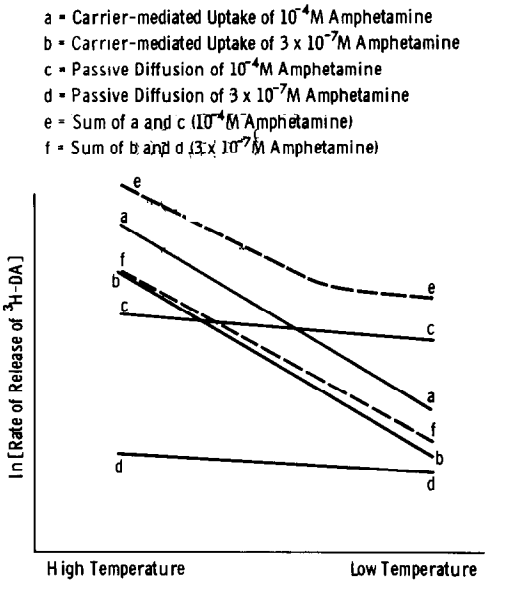


Fig. 13. Hypothetical model for amphetamine-induced release of [$^3$H]DA. Solid lines represent the [$^3$H]DA release by amphetamine which enters into neurons either by passive diffusion or by a carrier-mediated uptake process. Dashed lines indicate the total release of [$^3$H]DA produced by amphetamine which has entered both passively and by carrier-mediated transport. The dashed lines also represent data obtained by experimentation ["e" is the release produced by $10^{-4}$ M amphetamine (Fig. 12); "f" is the release produced by $3 \times 10^{-7}$ M amphetamine (Fig. 11)].

of [$^3$H]DA produced by $3 \times 10^{-7}$ M amphetamine primarily involves accelerated exchange diffusion (Fig. 13, line b). Therefore, the overall release of [$^3$H]DA produced by amphetamine would be similar to that of the release produced by amphetamine which has entered into neurons by a carrier system. Consequently, a straight Arrhenius plot would be obtained (Fig. 13, line f). However, at high concentrations of amphetamine ($10^{-4}$ M), the carrier is probably saturated by the drug, and a greater proportion of amphetamine enters the neuron by passive diffusion. Thus, at higher amphetamine concentrations, the release of [$^3$H]DA produced by amphetamine that has entered the neurons by passive diffusion begins to play a more dominant role in the total release process. In this case, the total release of [$^3$H]DA produced by amphetamine is the sum (Fig. 13, line e) of the rates of both accelerated diffusion (Fig. 13, line a) and passive diffusion of amphetamine (Fig. 13, line c).

In the present study, a broken Arrhenius plot was observed with $10^{-4}$ M amphetamine. The higher $Q_{10}$ observed between 30° and 37° may be explained by the carrier-mediated entry of amphetamine, whereas the low $Q_{10}$ observed between 23° and 30° may suggest a passive diffusion of amphetamine. This would indicate that facilitated diffusion is not a predominant mechanism at the lower temperatures. A similar explanation may also be applicable for unlabelled DA-induced release of [$^3$H]DA. The rather subtle changes in the slopes of Arrhenius plots with decreas-

ing temperature are possibly due to the lower lipid solubility of unlabelled DA relative to amphetamine. In other words, the activation energy required for the passive transport of unlabelled DA across the neuronal membrane would most likely be higher than for amphetamine.

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