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# Compartmentation of Dopamine in Rat Striatum

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SUMMARY

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Dopamine in the rat corpus striatum declines biphasically after intraperitoneal injection of 0.81 nmole/kg of α-methyl-*p*-tryosine methyl ester. This confirms the results of Javoy and Glowinski [(1971) *J. Neurochem.*, **18:** 1305–1311]. The rapid initial decline in striatal dopamine is coincident with *p*-hydroxyamphetamine and *p*-hydroxynorephedrine concentrations of 1.2 and 8.2 nmoles/g of brain, respectively. Similar concentrations are present in the striatum. *p*-Hydroxyamphetamine and *p*-hydroxynorephedrine may be responsible for the fast initial decline in striatal dopamine after treatment with α-methyl-*p*-tyrosine methyl ester, because these compounds change the kinetics of striatal dopamine, presumably by increasing its efflux. Moreover, the conversion index of striatal labeled tyrosine to striatal dopamine is inhibited only 53 % between 5 and 15 min after injection of α-methyl-*p*-tyrosine methyl ester. We propose a new method for calculating the turnover rate of striatal dopamine, based on normalized plots of the change with time of the specific activity of striatal tyrosine and dopamine after injection of tracer amounts of [$^3$H]tyrosine. The transformation constant ($\tau$) is defined by the fractional rate constant of tyrosine ($k_{Tyr}$) and is calculated as equal to $1/k_{Tyr}$. This kinetic analysis yields an estimate of the striatal dopamine turnover rate in agreement with values estimated by other methods. The experimental data are incompatible with a two-compartment model of dopamine: a functional compartment (26 % of the store) and a main storage compartment (74 % of the store) with $k$ values of 4.6 and 0.34 $hr^{-1}$, respectively, as proposed by Javoy and Glowinski. Calculations show that the 0.34 $hr^{-1}$ rate for a single striatal dopamine compartment exceeds the limits imposed by the change with time of striatal dopamine specific radioactivity after a tracer injection of [$^3$H]tyrosine.

## INTRODUCTION

The biphasic decline in striatal dopamine concentrations observed in rats receiving a single intraperitoneal injection (0.81 mmole/kg) of the tyrosine hydroxylase inhibitor α-methyltyrosine methyl ester (1) has been interpreted by Javoy and Glowinski (2) to indicate that in dopaminergic nerve terminals there are two distinct storage forms of dopamine. From this biphasic decline they inferred that the functional pool of transmitter includes about 26% of the total striatal dopamine, and they calculated that this pool has a fractional rate consant ($k$) of 4.6 $hr^{-1}$. They also proposed that the main storage pool includes the remaining striatal dopamine, which has a $k$ value of 0.34 $hr^{-1}$.

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The present paper reports a kinetic analysis and a new method for calculating the conversion rate of radioactive tyrosine into dopamine in rat striatum. This analysis reveals that the compartmentation of striatal dopamine proposed by Javoy and Glowinski (2) is not consistent with the experimental data. We also provide data on the concentrations of α-methyltyrosine and its metabolites in brain and striatum. We found that the high concentrations of *p*-hydroxyamphetamine and *p*-hydroxynorephedrine formed from MT[2] increase the release of striatal dopamine. Moreover, during the 15 min after MT injection, this amino acid inhibits the conversion of radioactive tyrosine to dopamine by a smaller extent than at 40 min. These data suggest that the biphasic decline in striatal dopamine reported by Javoy and Glowinski (2) after MT treatment may be related both to incomplete inhibition of brain tyrosine hydroxylase and to an increased release of dopamine during the first 20 min after an intraperitoneal dose of MT; subsequently the tyrosine hydroxylase inhibition is greater but not complete. The assumption that this intraperitoneal dose of MT causes immediate and complete inhibition of tyrosine hydroxylase is not valid; therefore MT cannot be used to study the compartmentation of dopamine in striatal nerve terminals.

## METHODS

The experiments were carried out with Sprague-Dawley male rats, weighing about 200 g, furnished by Zivic Miller, Allison Park, Pa. The animals were kept in air-conditioned rooms at about 20° for at least 3 days before the experiments. DL-α-Methyl-*p*-tyrosine methyl ester HCl (Regis Chemical Company) was dissolved to yield a solution containing in 1 ml the dose to be injected into a 200-g rat. The dose injected intraperitoneally was identical with that used by Javoy and Glowinski (2) (0.81 mmole/kg).

Tritiated L-3,5-tyrosine (specific activity, 42 Ci/mmole), obtained from New England Nuclear Corporation, was injected intravenously (0.5 mCi/kg in 5 ml), and the rats were killed at various times with a microwave source (1.5 KW operating at a frequency of 2.45 GHz). Details on the radiation source are described by Wang and Craig (3). When the rat skull is exposed for 2 sec to this irradiation, the enzymes involved in catecholamine metabolism are inactivated.[3] The striatum was dissected as described by Javoy and Glowinski (2).

### *Chemical Procedures*

The concentrations of MT and its metabolites in brain were assayed as follows. We homogenized brain tissue in 4 volumes of 0.4 N perchloric acid containing 0.05% sodium metabisulfite. After centrifugation at 12,000 × *g* for 10 min at 4°, we removed 4.7 ml of the supernatant fluid and added 0.3 ml of 10 M potassium acetate. After a second centrifugation, the supernatant fluid (4 ml) was placed on a Dowex 50-X4 column (200–400 mesh, 25 mm × 26 mm²) prepared as described by Haggendal (4). This column will be referred to as column A.

We collected the effluent and the column A eluate with 0.1 M sodium acetate buffer, pH 4.5, containing 0.1% disodium edetate. These two fractions were combined and brought to pH 8.3 with 0.75 ml of 3 M tromethamine. Alumina (Woelm neutral, grade 1), about 500 mg [prepared as described by Crout (5)], that had been washed with 5 ml of 0.1 M disodium edetate was added to the two combined fractions eluted from column A. The samples were manually shaken for 10 min and then centrifuged at 1000 × *g* for 10 min. The supernatant fraction (7.5 ml) was titrated to pH 1.5, and 7 ml of this solution were placed on another Dowex 50-X4 column (column B) similar to column A. We discarded the sample effluent and the effluent after the addition of 5 ml of water and 8 ml of 0.5 N hydrochloric acid. MT was eluted by adding 7 ml of 0.1 M tribasic sodium phosphate to column B and was assayed by forming a fluorophor with 1-nitroso-2-naphthol as described by Uden-

[2] The abbreviations used are: MT, α-methyl-*p*-tyrosine methyl ester; HA, *p*-hydroxyamphetamine; HNE, *p*-hydroxynorephedrine.

[3] Unpublished observations (Guidotti, A. and Costa, E.).

friend (6). Recovery of MT added to tissue homogenates was 65%.

Column A was rinsed with 10 ml of 0.1 M sodium acetate buffer (pH 6.0) and 15 ml of 0.4 N HCl; both eluates were discarded. Column A was successively eluted with 8 ml of 1 N HCl and with 4 ml of sodium phosphate solution (80 ml of 0.4 M $Na_3PO_4$ and 20 ml of 0.4 M $Na_2HPO_4$, pH 11.2). The 1 N HCl eluate, which contained *p*-hydroxynorephedrine, was lyophilized, and the residue was dissolved in 2 ml of 0.4 M tribasic sodium phosphate. This reconstituted fraction and the sodium phosphate eluate from column A, which contained *p*-hydroxyamphetamine, were assayed for both products by forming a fluorophor with 1-nitroso-2-naphthol as described by Udenfriend (6). The fluorescence was read in an Aminco-Bowman spectrophotofluorometer at 450 nm (excitation) and 560 nm (emission) (uncorrected). The recoveries of HA and HNE were 85% and 70%, respectively.

Specific activities of tyrosine and dopamine were analyzed according to Neff *et al.* (7); dopa specific activity was measured according to Costa (8).

### *Analysis of Data*

*Decline in dopamine level after MT.* The rate of change in striatal dopamine concentration with time was measured by converting dopamine concentrations into logarithmic values (base 10), which were used directly to measure the regression coefficient by lognormal analysis.

The regression coefficient of the decline in striatal dopamine in groups of rats killed at various times after MT treatment was calculated for sequential groups, each including a constant number of subsets. The calculations were done using two sequences: the subset of three, the initial subset, included animals killed 0, 5, and 10 min after MT; and the subset of four, animals killed at 0, 5, 10, and 15 min in the initial subset.

Statistical comparison between the regression line for a subset and the regression line of the entire set was performed as follows:

$$t = \frac{b_1 - b_2}{\sqrt{sp^2[(1/\Sigma x_1^2) + (1/\Sigma x_2^2)]}}$$

where

$$sp^2 = \frac{\{\Sigma y_1^2 - [(\Sigma x_1 y_1)^2/\Sigma x_1^2]\} + \{\Sigma y_2^2 - [(\Sigma x_2 y_2)^2/\Sigma x_2^2]\}}{(n_1 - 2) + (n_2 - 2)}$$

where $b_1$ and $b_2$ are the slopes of the subset and of the entire set, respectively, $t$ is the significant limit, and $sp^2$ is the best estimate of the variation in the regression analysis.

*Calculation of dopamine turnover rate from changes of its specific activity with time.* The current understanding of the process involved in dopamine biosynthesis supports the assumption that striatal tyrosine is converted into striatal dopamine as follows:

$$\text{Tyr} \xrightarrow[k_{\text{Tyr}}]{} \text{dopa} \xrightarrow[k_{\text{dopa}}]{} \text{DA} \xrightarrow[k_{\text{DA}}]{}$$

In the model Tyr represents the tyrosine pool in the striatum, and dopa and DA, the pools of 3,4-dihydroxyphenylalanine and dopamine, respectively. The process of conversion of tyrosine to dopa to dopamine is characterized by the fractional rate constants $k_{\text{Tyr}}$, $k_{\text{dopa}}$, and $k_{\text{DA}}$. Since the pool of striatal dopa is small (about 0.5% of that of dopamine), $k_{\text{dopa}}$ is much larger than $k_{\text{Tyr}}$ and $k_{\text{DA}}$; moreover, the specific activity of dopa 3–50 min after labeling is similar to that of tyrosine (Table 1). These considerations suggest that it is mathematically permissible to calculate the turnover rate of striatal dopamine from the changes with time in the specific activities of striatal tyrosine and dopamine. The differential equation for the conversion of dopa to dopamine at the steady state is

$$\frac{d[\text{DA}]}{dt} = k_{\text{dopa}}[\text{dopa}] - k_{\text{DA}}[\text{DA}] = 0 \quad (1)$$

After labeling with radioactive tyrosine the following relationship is valid:

$$\frac{dS_{\text{DA}}}{dt} = k_{\text{DA}}(S_{\text{dopa}} - S_{\text{DA}}) \quad (2)$$

where $S_{\text{DA}}$ and $S_{\text{dopa}}$ are the specific radioactivities of dopamine and dopa.

TABLE 1

*Steady-state and specific activities of striatal tyrosine and dopa at various times after* L[3,5-$^3$H]*tyrosine*

Each value was obtained by pooling the striata of eight rats. Each rat received 0.4 mCi/kg of L-[3,5-$^3$H]tyrosine intravenously at zero time.

| Time after labeling | Tyrosine | | Dopa | |
|---|---|---|---|---|
| | Concentration | Specific activity | Concentration | Specific activity |
| *min* | *nmoles/g* | *dpm/nmole* | *nmole/g* | *dpm/nmole* |
| 3 | 72 | 5420 | 0.29 | 4990 |
| 6 | 80 | 5140 | 0.29 | 4850 |
| 9 | 73 | 4670 | 0.31 | 3910 |
| 12 | 77 | 4400 | 0.29 | 4100 |
| 15 | 82 | 3480 | 0.34 | 3600 |
| 18 | 81 | 2990 | 0.33 | 2790 |
| 30 | 74 | 2120 | 0.29 | 2000 |
| 50 | 70 | 750 | 0.31 | 900 |

The question arose whether $S_{Tyr}$ could be substituted for $S_{dopa}$. It is obvious that this substitution can be made only if $S_{Tyr} = S_{dopa}$, as suggested by the data in Table 1. Once we satisfied this requirement, we followed the kinetic approcach described below.

If the specific activity of tyrosine ($S_{Tyr}$) declines exponentially:

$$S_{Tyr}(t) = S_{Tyr}(0)\, e^{-k_{Tyr}t} \qquad (3)$$

Then the specific activity of dopamine ($S_{DA}$) as derived by Neff *et al.* (9) will be

$$S_{DA}(t) = S_{Tyr}(0)\, \frac{k_{DA}}{k_{DA} - k_{Tyr}} \left(e^{-k_{Tyr}t} - e^{-k_{DA}t}\right) \qquad (4)$$

Defining $\tau$ as the turnover time of tyrosine, $1/k_{Tyr} = \tau$, and taking $\tau$ as a unit of time, we can now use $\tau$ as a transformation constant by plotting the logarithm (base 10) of $S_{DA}$ as a function of $t/\tau$ on the $x$ axis. $S_{NTyr}$ and $S_{NDA}$ will now denote the transformed (normalized) values of $S_{DA}$ and $S_{Tyr}$; these are pure numbers without dimension.

Defining

$$S_{NTyr}(t/\tau) = \frac{S_{Tyr}(t)}{S_{Tyr}(0)}$$

$$S_{NDA}(t/\tau) = \frac{S_{DA}(t)}{S_{Tyr}(0)}$$

$$k_{NDA} = \frac{k_{DA}}{k_{Tyr}}$$

Eqs. 3 and 4 can be rewritten as

$$S_{NTyr}(t/\tau) = e^{-t/\tau} \qquad (5)$$

and

$$S_{NDA}(t/\tau) = \frac{k_{NDA}}{k_{NDA} - 1} \left(e^{-t/\tau} - e^{-k_{NDA}(t/\tau)}\right) \qquad (6)$$

When $t/\tau = 1$,

$$S_{NTyr}(t/\tau) = \frac{1}{2.718281\ \cdots} = 0.367$$

One can draw a family of curves of $S_{NDA}$ $(t/\tau)$ using $k_{NDA}$ as a parameter, based upon a time scale of $t/\tau$ on the $x$ axis. Table 2 lists values of $S_{NDA}$ calculated from Eq. 6 after varying $k_{NDA}$ but maintaining $k_{Tyr}$ at 2.4 hr$^{-1}$, which was the value found in these experiments.

The normalized experimental values of $S_{DA}$ ($S_{NDA}$) can be fitted to a curve defined by a given $k_{NDA}$. Often the fit is not perfect, but in such an instance the data point of $S_{NDA}$ can be bracketed with a high degree of accuracy by two curves derived from Eq. 6, each curve characterized by its own $k_{NDA}$. The difference between these two $k_{NDA}$ values describes the uncertainty of the normalized fractional rate constant.

By definition

$$k_{NDA} = \frac{k_{DA}}{k_{Tyr}}$$

thus $k_{DA}$ can be calculated from

$$k_{DA} = (k_{NDA})(k_{Tyr}) \qquad (7)$$

The turnover of dopamine ($TR_{DA}$) is

$$TR_{DA} = k_{DA}[DA] \qquad (8)$$

## RESULTS

*Decline in striatal dopamine after intraperitoneal injection of MT.* We have con-

TABLE 2

*Calculated values of $S_{NDA}$ $(t/\tau)$ for various theoretical values of $k_{NDA}$*

| $t/\tau$ | $S_{NDA}(t/\tau)$ for $k_{NDA}$ | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.08 | 0.10 | 0.12 | 0.15 | 0.17 | 0.20 | 0.25 |
| 0.1 | 0.0076 | 0.0095 | 0.0114 | 0.0142 | 0.0160 | 0.0188 | 0.0235 |
| 0.2 | 0.0143 | 0.0179 | 0.0215 | 0.0265 | 0.0303 | 0.0355 | 0.0442 |
| 0.3 | 0.0205 | 0.0255 | 0.0305 | 0.0380 | 0.0429 | 0.0502 | 0.0623 |
| 0.5 | 0.0308 | 0.0383 | 0.0457 | 0.0567 | 0.0639 | 0.0746 | 0.0920 |
| 0.7 | 0.0390 | 0.0484 | 0.0571 | 0.0712 | 0.0801 | 0.0932 | 0.1140 |
| 1.0 | 0.0483 | 0.0597 | 0.0708 | 0.0870 | 0.0974 | 0.1130 | 0.1370 |
| 1.2 | 0.0528 | 0.0651 | 0.0770 | 0.0942 | 0.1050 | 0.1210 | 0.1470 |
| 1.5 | 0.0577 | 0.0708 | 0.0835 | 0.1020 | 0.1130 | 0.1290 | 0.1550 |
| 2.0 | 0.0623 | 0.0759 | 0.0888 | 0.1070 | 0.1180 | 0.1340 | 0.1570 |
| 2.5 | 0.0640 | 0.0774 | 0.0898 | 0.1070 | 0.1170 | 0.1310 | 0.1510 |
| 3.0 | 0.0641 | 0.0789 | 0.0883 | 0.1040 | 0.1130 | 0.1250 | 0.1410 |
| 4.0 | 0.0616 | 0.0724 | 0.0819 | 0.0936 | 0.1000 | 0.1080 | 0.1170 |
| 5.0 | 0.0577 | 0.0666 | 0.0739 | 0.0822 | 0.0862 | 0.0903 | 0.0933 |
| 6.0 | 0.0536 | 0.0607 | 0.0660 | 0.0713 | 0.0773 | 0.0747 | 0.0736 |
| 7.0 | 0.0496 | 0.0551 | 0.0587 | 0.0616 | 0.0621 | 0.0614 | 0.0576 |

firmed the report by Javoy and Glowinski (2) that the intraperitoneal injection of MT causes a biphasic decline in the striatal concentrations of dopamine (Table 3). Sequential analysis of the dopamine regression coefficient, $b = k_{DA}/0.434$ (10), reported in Table 3, shows that $k_{DA}$ is significantly greater during the first subset ($p < 0.05$) when the data are ranked in a series formed by groups of three (0, 5, and 10 min) than in any other successive subset of the same series; moreover, $k_{DA}$ during the first 10 min is greater than that calculated from the extrapolated regression during the 2 hr after intraperitoneal injection of MT. However, when the regression coefficient is measured using the series in which each subset includes four values (0, 5, 10, and 15 min), $k_{DA}$ is no longer different from the $k_{DA}$ calculated from the slope ($b$) for the whole set, extrapolating to zero time the exponential decline from 15 to 120 min. We also measured the concentrations of MT in brain (Table 4) at various times after intraperitoneal injections of this amino acid. Table 4 also reports the brain concentrations of *p*-hydroxyamphetamine and *p*-hydroxynorephedrine. These data show that

TABLE 3

*Concentrations of striatal dopamine at various times after single intraperitoneal injection of α-methyl-p-tyrosine methyl ester HCl*

MT (0.81 mmole/kg) was injected intraperitoneally at zero time. Values are the means ± standard errors of determinations on four rats.

| Time after MT | Dopamine | Sequential analysis (subset with 3 time values) | | Sequential analysis (subset with 4 time values) | | $k_{DA}$ (0–120 min)[a] |
|---|---|---|---|---|---|---|
| | | $k_{DA}$ | $t$ | $k_{DA}$ | $t$ | |
| *min* | *nmoles/g* | $hr^{-1}$ | | $hr^{-1}$ | | $hr^{-1}$ |
| 0 | 64 ± 2.7 | | | | | |
| 5 | 60 ± 1.5 | | | | | |
| 10 | 56 ± 1.9 | 0.85 | 2.62[b] | | | |
| 15 | 56 ± 2.7 | 0.45 | 0.95 | 0.60 | 0.84 | |
| 20 | 52 ± 2.9 | 0.29 | 1.08 | 0.45 | 0.92 | |
| 60 | 42 ± 0.7 | 0.38 | 0.84 | 0.30 | 0.85 | |
| 120 | 35 ± 1.6 | 0.28 | 1.06 | 0.27 | 1.08 | 0.30 |

[a] This value was obtained from $b$ of the exponential decline observed from 15 to 120 min, extrapolated back to zero time ($\log y = 1.783 - 0.12654x$).

[b] $p < 0.05$ when each subset is compared with $K_{DA}$ of the entire set.

TABLE 4

*Concentrations of α-methyl-p-tyrosine, p-hydroxynorephedrine, and p-hydroxyamphetamine in rat brain and striatum after administration of MT (0.81 mmole/kg)*

Values are means ± standard errors of the numbers of animals shown in parentheses.

| Time after injection | MT | | HA | | HNE | |
|---|---|---|---|---|---|---|
| | Brain | Striatum | Brain | Striatum | Brain | Striatum |
| *min* | *nmoles/kg* | | *nmoles/kg* | | *nmoles/kg* | |
| 5 | 10 ± 3 (4) | | 1.2 ± 0.4 (4) | | | |
| 10 | 25 ± 8 (4) | 32 (10) | 0.8 ± 0.3 (4) | 1.2 (10) | 8.2 ± 1.1 (3) | 14 (10) |
| 20 | 67 ± 21 (4) | | 1.1 ± 0.6 (4) | | | |
| 40 | 95 ± 18 (4) | 84 (10) | 0.4 ± 0.1 (4) | 0.2 (10) | 1.6 ± 0.4 (3) | 1.5 (10) |

the concentrations of MT increase 9-fold during the time interval included in our studies. The values compatible with 87% inhibition of tyrosine hydroxylase (11) are achieved only 20 to 40 min after MT injection. The concentrations of HA oscillate around 1 nmole/g during the experimental time period, whereas the concentrations of HNE are about 10 times those of HA at 10 min and about 4 times the HA levels 40 min after MT injection. We also measured the concentrations of MT, HA, and HNE in 10 striata of rats killed 10 and 40 min after the intraperitoneal injection of MT. These results confirm that the concentrations of these compounds in striatum are similar to those found in the rest of the brain tissues.

*Conversion of L-[3,5-$^3$H]tyrosine to dopamine at various times after intraperitoneal injection of MT and intraventricular injection of HA and HNE.* We measured the specific activities of tyrosine and dopamine in striata of rats receiving NaCl or MT. The data reported in Table 5 show that 5 min after MT there is inhibition of the uptake of radioactive tyrosine in striatum but that the conversion of tyrosine to dopamine is inhibited by only 53%. This conversion is further inhibited at 40 min but even at this time inhibition is not complete (87% inhibition).

To decide whether the presence of HA and HNE had any effect on the kinetics of dopamine in striatum, we implanted a polyethylene cannula (PE 10) in the lateral brain ventricles of 12 rats. Two days later the rats were given an intravenous injection of radioactive tyrosine, and 40 min later

TABLE 5

*Conversion index (CI) of striatal dopamine 5 and 40 min after MT*

MT (0.81 mmole/kg) was injected intraperitoneally at zero time; L-[3,5-$^3$H]tyrosine (1 mCi/kg) was injected intravenously either 5 or 40 min later. Ten minutes after labeling, the two groups of rats were killed. Another group of rats received only the label. Values are means ± standard errors of determinations on four rats.

| Time after MT | Dopamine | Tyrosine | CI[a] |
|---|---|---|---|
| *min* | *dpm/nmole* | | *nmoles/g/hr* |
| 0 | 249 ± 27 | 4850 ± 140 | 18 ± 1.4 |
| 5–15 | 77 ± 23[b] | 2760 ± 50[b] | 9.5 ± 3.0[b] |
| 40–50 | 47 ± 4[b] | 5367 ± 730 | 2.5 ± 0.8[b] |

[a] The conversion index was estimated as follows:

$$\text{Dopamine (nmoles/g/hr)} = \frac{\text{dopamine (dpm/g) at 10 min}}{\text{tyrosine (dpm/nmole) at 10 min}} \times 6.$$

[b] $p < 0.01$ compared with rats not receiving MT.

they received NaCl, HA, or HNE intraventricularly in amounts comparable to those present in striatum after MT injections (see Table 4). The rats were killed 20 min later, and the specific activities of striatal tyrosine and dopamine were measured. The data reported in Table 6 show that HA and HNE failed to change the steady-state concentrations of tyrosine and dopamine and the specific activity of tyrosine. However, the specific activity of striatal dopamine was significantly increased by these two compounds. Since the

TABLE 6

*Effects of HA and HNE injected intraventricularly 40 min after* L-[3,5-$^3$H]*tyrosine on specific activities of striatal tyrosine and dopamine*

HA (10 nmoles), HNE (10 nmoles), or NaCl (10 μl) was injected through a cannula implanted into the lateral ventricule after the intravenous administration of L-[3,5-$^3$H]tyrosine. The rats were killed 20 min after the intraventricular injection. Values are means ± standard errors of determinations on four rats, and were determined 60 min after labeling.

| Drug | Tyrosine | | Dopamine | |
|---|---|---|---|---|
| | concentration | specific activity | concentration | specific activity |
| | *nmoles/g* | *dpm/nmole* | *nmoles/g* | *dpm/nmole* |
| NaCl | 85 ± 3.9 | 1450 ± 50 | 65 ± 3.0 | 1644 ± 100 |
| HA | 84 ± 3.1 | 1580 ± 170 | 63 ± 1.3 | 1960 ± 120[a] |
| HNE | 83 ± 4.3 | 1290 ± 150 | 63 ± 1.5 | 2140 ± 150[a] |

[a] $p < 0.05$.

steady state was not changed, these data suggest that HA and HNE increased the release of striatal dopamine.

*Kinetic analysis of conversion of striatal tyrosine to dopamine.* In Table 7 are reported $S_{DA}$ and $S_{Tyr}$ at various times after the intravenous injection of L-[3,5-$^3$H]tyrosine. The data were obtained in two different experiments, using 0.5 and 0.4 mCi/kg intravenous doses of the labeled amino acid. Calculations using the kinetic approach described under METHODS show that in these two experiments the data generate similar $k_{DA}$ values.

As shown in Fig. 1, the $\tau$ value calculated from the slope of $S_{Tyr}$ is 25 min. Thus in our plot 1 unit of $t/\tau$ is equal to 25 min. The data plotted in Fig. 1 show that $S_{NTyr}(t/\tau)$ declines exponentially while on the $x$ axis $t/\tau$ changes from 0.4 to 2. Above this value of $t/\tau$, $S_{NTyr}(t/\tau)$ deviates from linearity. However, it is important to note that the exponential mode of decline is preserved until $S_{NTyr}(t/\tau)$ intersects $S_{NDA}(t/\tau)$. The data points ($S_{NDA}$) are fitted within two curves generated by Eq. 6, which have $k_{NDA}$ values between 0.12 and 0.15. Thus, as shown in Fig. 1, the $k_{DA}$ (calculated from Eq. 7) varies between 0.36 and 0.29 hr.$^{-1}$ Considering that the concentration of striata dopamine is 64 ± 2.7 nmoles/g (Table 3), the turnover rate calculated from Eq. 8 can be estimated as 18–23 nmoles/g/hr.

We then considered whether $S_{NDA}(t/\tau)$ could eventually be fitted by the sum of the two curves with $k_{DA}$ values of 4.6 and 0.34 hr$^{-1}$, $k_{DA}$ respectively. These represent the $k_{DA}$ values of the two pools of striatal dopamine, each including 26% and 74% of the striatal transmitter, respectively, as proposed by Javoy and Glowinski (2). In Fig. 2 we report the theoretical values for $S_{NDA}(t/\tau)$ derived from our experimental $k_{Tyr}$, considering the compartmentation proposed by Javoy and Glowinski (2) as

TABLE 7

*Striatal dopamine and tyrosine specific radioactivities at various times after intravenous injection of* L-[3,5-$^3$H]*tyrosine*

Rats received 0.5 mCi/kg of L-[3,5-$^3$H]tyrosine (A) or 0.4 mCi/kg (B). Each value is the average of eight striata. The concentrations of dopamine (63 ± 4.2 nmoles/g) and tyrosine (73 ± 5.8 nmoles/g) remained constant in both experiments. The $k_{DA}$ for experiment A was 0.29–0.34 hr$^{-1}$, and that for experiment B was between 0.28–0.36 hr$^{-1}$.

| Time after [$^3$H]tyrosine injection | $S_{DA}$ | | $S_{Tyr}$ | |
|---|---|---|---|---|
| | A | B | A | B |
| *min* | *dpm/nmole* | | *dpm/nmole* | |
| 3 | | 83 | | 6795 |
| 6 | 189 | 161 | 8847 | 5134 |
| 9 | 358 | 255 | 6304 | 4832 |
| 15 | 447 | 350 | 5903 | 3473 |
| 30 | 708 | 622 | 3342 | 2114 |
| 60 | 957 | | 994 | |
| 90 | 905 | | 514 | |
| 120 | 727 | | 296 | |
| 180 | 580 | | 184 | |

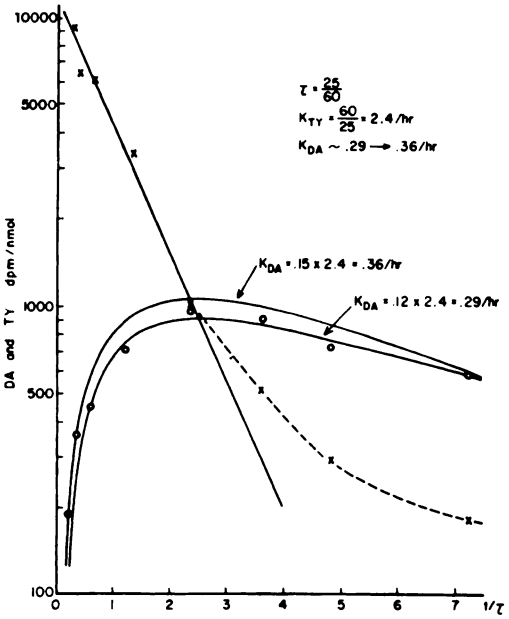


FIG. 1. *Normalized plot of specific activities of striatal dopamine, $S_{NDA}(t/\tau)$, and tyrosine, $S_{NTY}(t/\tau$*
The constant of transformation is $\tau = 1/k_{Tyr}$, where $k_{Tyr}$ is 2.4 $hr^{-1}$. The values, unless otherwise indicated, are expressed in minutes. O——O, $S_{DA}$; ×- - -×, $S_{Tyr}$.

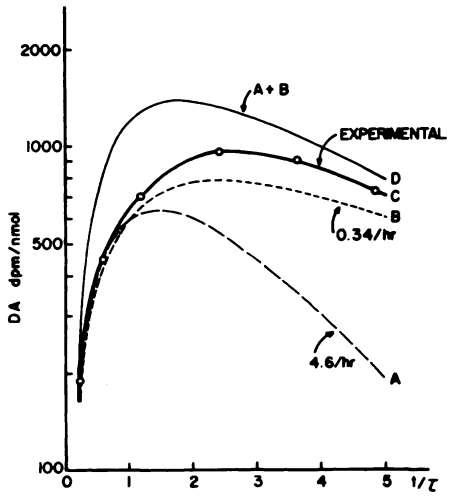


FIG. 2. *Calculated values of $S_{NDA}$, assuming compartmentation of striatal dopamine (DA) proposed by Javoy and Glowinski* (2).
One compartment is proposed to have a $k_{DA}$ of 4.6 $hr^{-1}$, and the other, a $k_{DA}$ of 0.34 $hr^{-1}$; the first functional compartment is 26% of the total dopamine in striatum, and the other includes 74% of the total dopamine. Data are plotted as $S_{NDA}(t/\tau)$, where $\tau = 1/k_{Tyr}$. Curve $A$ = $S_{NDA}$ in the "functional" compartment ($k_{DA}$ = 4.6 $hr^{-1}$, including 26% of striatal dopamine); $B$ = $S_{NDA}$ of the "main storage" compartment ($k_{DA}$ = 0.34 $hr^{-1}$, including 74% of striatal dopamine); $C$ = experimental $S_{NDA}$ according to Fig. 1; $D = A + B$, where data are plotted as $S_{NDA}(t/\tau)$, with $\tau = 1/k_{Tyr}$.

operative in striatum. Neither of the two curves ($A$ and $B$ in Fig. 2) nor the sum of the two ($D$) is compatible with the experimental data points ($C$).

## DISCUSSION

When principles of steady-state kinetics are applied to the decline of neurotransmitter concentrations after inhibition of synthesis in order to elucidate the compartmentation of the transmitter, the inhibition must be complete and instantaneous, and the transmitter storage must not be under the influence of other perturbing agents (12). However, the data reported in Tables 4–6 indicate that both these requirements were not fulfilled by the experimental conditions used by Javoy and Glowinski (2). Indeed, under these conditions one obtains a biphasic decline in striatal dopamine, although we believe that this mode of change in striatal dopamine concentration cannot be attributed exclusively to its kinetic compartmentation. Udenfriend (11), working with the isolated, perfused guinea pig heart, has reported that a MT concentration of 87 μM causes 87% inhibition of catecholamine biosynthesis and that a MT concentration of 39 μM causes inhibition of only 61%. Table 4 reports that at 5 min after injection the brain concentration of MT is 10 μM. Thus, extrapolating from Udenfriend's data (11) during the first 5 min after the intraperitoneal injection of 0.81 mmole of MT, these concentrations of MT should result in incomplete inhibition of tyrosine hydroxylase (less than 61%). Moreover, according to the data of Table 4, inhibition greater than 87% may occur only after 40 min postinjection. The validity of these extrapolations is supported by the data in Table 5. The conversion index of radioactive tyrosine into striatal dopamine is inhibited about 53% between 5 and 15 min after MT

and about 87% between 40 and 50 min after MT. It is precisely from the fast rate of dopamine decline between 0 and 20 min that Javoy and Glowinski (2) proposed the existence of a rapidly turning over pool of striatal dopamine. But the data reported in Table 4 show that substantial concentrations of HA and HNE can be found in brains of rats 10 min after receiving MT intraperitoneally. These two compounds are able to perturb the dynamics of catecholamine storage in peripheral nerve terminals (13), causing a rapid decline in catecholamine concentrations (14). The data reported in Table 6 show that intraventricular injection of either HA or HNE in amounts comparable to those present in striatum during 10 min following 0.81 mmoles/kg of MT intraperitoneally (Table 4) perturbs the kinetics of dopamine in striatum. The data reported in Table 6 appear to be consistent with the view that the efflux of dopamine from striatal nerve endings is increased by HA or HNE. Presumably, when the two compounds are present simultaneously, their action is synergistic. We suggest that the slow onset of tyrosine hydroxylase inhibition and the presence of HA and HNE, which releases catecholamines from storage, account for the biplasic decline in striatal dopamine. From the data reported in Table 4, it appears that brain and striatal concentrations of MT increase 5–40 min after the injection of this amino acid, but the brain and striatal concentrations of HA and HNE decline during the same time period. This would be consistent with the view that high concentrations of MT inhibit its own decarboxylation, as reported similarly for $\alpha$-methyldopa (15, 16). Appropriate experiments should be performed to test this possibility. Since MT is an inadequate research tool to measure the compartmentation of striatal dopamine, we resorted to the kinetic approach described under METHODS.

The data reported in Fig. 1 and Table 7 allow us to calculate a turnover rate of striatal dopamine between 18 and 23 nmoles/g/hr. These values are consistent with 19.2 nmoles/g/hr, which is the value calculated from the data of Table 3, using the extrapolated $k_{DA}$ value of 0.30 $hr^{-1}$. Moreover, they are consistent not only with values obtained with another method published by this laboratory (17) but with the turnover rate of 16.9 nmoles/g/hr calculated by Javoy and Glowinski (2) for the main storage of striatal dopamine. These considerations indicate that inhibition of dopamine synthesis by injection of MT can be used to estimate the turnover rate of striatal dopamine if the values are collected during an extended time period. This approach minimizes the pitfalls created by the presence of HA and HNE during the first 20 min after injection (Table 4). Presumably HA and HNE release striatal dopamine (Table 6) by a mechanism similar to that reported for peripheral catecholamine stores (14). The question then arises: Are the conclusions proposed by Javoy and Glowinski (2) for the complex compartmentation of striatal dopamine valid? To answer this question, we performed the calculations reported in Fig. 2. We calculated how $S_{NDA}(t/\tau)$ should change if two pools with the characteristics described by Javoy and Glowinski (2) were operative in regulating the dynamics of striatal dopamine stores. The data show that $S_{NDA}(t/\tau)$ (curve $D$ in Fig. 2) would deviate substantially from the experimental values of $S_{NDA}(t/\tau)$ (curve $C$). This deviation would substantiate the view that the store of striatal dopamine may not be compartmentalized in the way proposed by Javoy and Glowinski (2): 26% turning over with a $k_{DA}$ of 4.6 $hr^{-1}$ and 74% turning over with a $k_{DA}$ of 0.34 $hr^{-1}$. We have therefore assumed that the size of the compartment turning over at 4.6 $hr^{-1}$ could be smaller than 26% of the total store. But using the change with time of $S_{NDA}$ reported in Fig. 1 (curve $C$ of Fig. 2), we could not calculate the presence of any other compartment of dopamine contributing to the kinetics of the system, which has a $k_{DA}$ faster than 0.34 $hr^{-1}$. If we try to fit the initial data points plotted in curve $C$ of Fig. 2 ($t/\tau < 1$) with a $k_{NDA}$ greater than 0.12, we find that this theoretical $S_{NDA}(t/\tau)$ is steeper than curve $C$ of Fig. 2, the experimental $S_{NDA}(t/\tau)$. This suggests that the corresponding $k_{DA}$ value would be in excess of what is allowed by the experimental data.

We attempted to reduce the size of the hypothetical compartment proposed by Javoy and Glowinski (2), and when we tested this possibility we found that even a pool of 5% of the total dopamine store would be too large to comply with the experimental data point reported in Fig. 1 and curve *C* of Fig. 2, when associated with 95% fo the store turning over with a $k_{DA}$ of 0.34 $hr^{-1}$.

In our calculations we made the assumption that $S_{Tyr}$ declines exponentially with time. This assumption represents the ideal case, in which the tyrosine pool receives a pulse injection of labeled tyrosine at the same reference time. As the tyrosine pool turns over in the steady state, its specific activity will follow an exponential decline. However, if some feedback loop exists in the biological system, some of the labeled tyrosine will find its way back into the pool. The net effect of such a feedback loop is a gradual deviation of $S_{Tyr}$ from exponential decay. The deviation from linearity increases with time.

This is exactly what we have observed. $S_{Tyr}$ can be closely approximated by a straight line on the semilogarithmic plot (i.e., $S_{Tyr}$ vs. $t/\tau$). At later points $S_{Tyr}$ assumes values higher than the straight line. We therefore used the initial $S_{Tyr}$ decline rate to determine the $\tau$ and hence the $k_{Tyr}$ value. We also feel it is reasonable to assume that the specific activity of that part of the tyrosine pool which is directly responsible for dopamine production was not significantly influenced by the feedback mechanism in the range of time $t/\tau \leqq 3$. It is therefore valid to use the procedure described under METHODS for $k_{DA}$ determination. The advantages of the present method over others previously proposed (12) are threefold: it allows us to estimate the uncertainty of fitting $k_{NDA}$ with experimental values; it facilitates comparisons of hypothetical compartmentations with experimental data (as shown in Fig. 2); and it satisfies the necessity of accounting for the precursor-product relationship within the limits of the assumptions made, at all times during the turnover rate measurement.

In conclusion, we do not deny that the compartmentation of striatal dopamine may actually be similar to the model proposed by Javoy and Glowinski (2), but suggest that the evidence for their interpretation is insufficient. Their interpretation must be questioned because of inherent methodological inconsistencies (see Tables 4 and 5) and evident contradictions with the experimental findings presented in this paper (see Fig. 2).

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