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Kinetics of Tyramine Transport and Permeation across Chromaffin-Vesicle Membranes†

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ABSTRACT: Tyramine permeates chromaffin-granule membranes via a reserpine-insensitive mechanism. The rate is unsaturable and increases with pH, indicating permeation of the unprotonated form of the amine. Reserpine-insensitive dopamine uptake is at least 10 times slower, consistent with dopamine's lesser lipophilicity. Dopamine is transported into chromaffin-granule membrane vesicles via a saturable, reserpine-sensitive, proton-linked mechanism. Tyramine inhibits dopamine transport with a K_i of 5–10 μM. Tyramine is not accumulated nearly as well as dopamine because inward transport is opposed by outward permeation. Nevertheless, the velocity of reserpine-sensitive tyramine transport can be deduced from the steady-state level of tyramine accumulation and the rate of permeation. V_{max} for tyramine transport is about one-third of the value for dopamine transport. Therefore, two aromatic hydroxyls are not needed for monoamine transport but are required for efficient accumulation and storage.

In studying the substrate specificity of membrane transport systems, one is generally limited to impermeant substrates although the behavior of more lipophilic molecules might be of interest. For example, catecholamine storage vesicles transport dopamine [2-(3,4-dihydroxyphenyl)ethylamine] but only poorly accumulate its more lipophilic analogue tyramine [2-(4-hydroxyphenyl)ethylamine]. It is thought that tyramine is transported, but because of its greater lipophilicity, it leaks back across the membrane in its unprotonated form (Scherman & Henry, 1980; Johnson et al., 1982). Because of this permeation, the rate of tyramine transport cannot be measured as the rate of net uptake. Nevertheless, it is important to measure the rate of tyramine transport for two reasons. First, it is necessary to clarify the substrate specificity of the amine translocator. Second, it may explain the well-known ability of tyramine to deplete vesicular catecholamine stores.

Transport into catecholamine storage vesicles has been most completely characterized in the chromaffin granules of the adrenal medulla (Carmichael, 1983). Chromaffin granules take up catecholamines via an H^+-linked transport system (Njus et al., 1981). The granule membrane has an inwardly directed H^+-translocating adenosinetriphosphatase (ATPase). Catecholamines are taken up by exchange diffusion for H^+ with an H^+/catecholamine stoichiometry of 2 (Knoth et al., 1980, 1981a; Phillips & Apps, 1980; Johnson et al., 1981). This exchange is thought to be mediated by a reserpine-sensitive amine translocator. The translocator has a rather broad specificity since chromaffin granules exhibit reserpine-sensitive uptake of epinephrine, norepinephrine, dopamine, and serotonin (DaPrada et al., 1975).

The amine translocator presumably catalyzes reserpine-sensitive tyramine transport as well [Figure 1(2)]. Tyramine can also cross the membrane by deprotonating [Figure 1(5)] and permeating in its unprotonated form [Figure 1(3)]. Tyramine fluxes via these two pathways establish a steady state in which inward transport is opposed by outward permeation. We have employed the following strategy to characterize the kinetics of tyramine transport and permeation across chromaffin-granule membranes. We can block transport using

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reserpine and study the kinetics of tyramine permeation in isolation. Then, in the absence of reserpine, we can measure the concentration gradients reached when inward transport and outward permeation are at steady state. Finally, from a mathematical analysis of these data, we can extract kinetic parameters for tyramine transport.

Materials and Methods

Chromaffin-granule membrane vesicles (ghosts) were prepared from bovine adrenal medulla as previously described (Njus & Radda, 1979). All experiments were completed within 12 h of slaughter. Protein was assayed by using biuret reagent (Casey et al., 1976).

Amine content of ghosts (Figures 2–4) was determined by collecting 1-mL samples under gentle suction on cellulose acetate filters (25-mm diameter, 0.45-μm pore size). Filters were washed 1 time with approximately 1 mL of ice-cold 250 mM KCl, 150 mM sucrose, and 40 mM Hepes[1] at the specified pH. The filters were then placed in 10 mL of scintillation fluid, and ^3H or ^{14}C activity was counted on a Beckman LS 100C liquid scintillation counter. A known amount of labeled monoamine was also counted and used to convert counts per minute into nanomoles.

Kinetics of tyramine uptake and efflux were analyzed by fitting data points (Figures 2 and 3) to an exponential function ($y = A + Be^{-Kt}$) using a least-squares method. The first 16 data points (first 8 time points) were used to obtain best-fitting values for A, B, and K. Since $dy/dt = -BKe^{-Kt}$, the initial velocity of uptake ($V_i = dy/dt$ at $t = 0$) was calculated from the uptake experiments as BK. For dopamine, V_i values were determined by fitting the first 10 data points (first 5 time points) to a straight line (Figure 4).

Steady-state tyramine, dopamine, and methylamine concentration gradients (Tables II–IV) were determined by measuring internal and external concentrations of [^{14}C]tyramine, [^{14}C]dopamine, or [^{14}C]methylamine as described by Casey et al. (1977). Ghosts (0.25 mL) were added to 0.25 mL of 250 mM KCl, 150 mM sucrose, and 40 mM Hepes at the indicated pH. Reserpine (5 or 10 mM) in acetone–ethanol (3:2) and 25 μL of 100 mM ATP–100 mM $MgSO_4$, pH 7, were added to the samples indicated. Reserpine-free samples received an equivalent volume of acetone–ethanol. Ten-microliter aliquots of 1 mM [^{14}C]dopamine (50 μCi/mL), 1 mM [^{14}C]tyramine (50 μCi/mL), or 1.33 mM [^{14}C]methylamine (67 μCi/mL) were added to the appropriate samples along with 1 μCi of ^3H_2O. After incubation for the specified time, samples were centrifuged for 20 min at 25000g at the incubation temperature. The pellets and supernatants were separated and processed as described by Casey et al. (1977). External and internal concentrations were calculated from ^{14}C activities in the supernatant and pellet, respectively, after making appropriate corrections for external aqueous volume trapped in the pellet. The internal volume was determined from the protein concentration assuming 3 μL/mg membrane protein.

Kinetic parameters for dopamine transport (Table V) were determined by the Lineweaver–Burk method as described by Knoth et al. (1981b). The initial rate of dopamine uptake was measured in ghosts (0.1–0.2 mg of protein/mL) suspended in 250 mM KCl, 150 mM sucrose, 2.5 mM $MgSO_4$, 2.5 mM ATP, and 40 mM Hepes at 25 °C. Uptake was assayed twice at each of nine dopamine concentrations ranging from 5 to 50 μM. A straight line fit to values of $1/V$ vs. $1/S$ by the least-squares method gave $-1/K_m$ from the x intercept and $1/V_{max}$ from the y intercept. In a parallel experiment done in the presence of 40 μM tyramine, $1/[K_m(1 + I/K_i)]$ was determined from the x intercept.

Reserpine, ATP, and unlabeled dopamine and tyramine were purchased from Sigma Chemical Co. All radioisotopes were obtained from New England Nuclear.

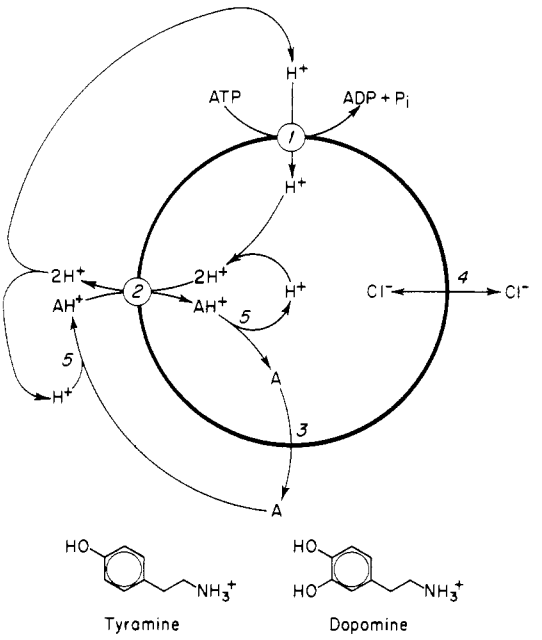

FIGURE 1: Summary of amine and ion fluxes in chromaffin-granule ghosts. (1) ATP-dependent proton translocation, (2) proton-linked amine transport, (3) permeation of unprotonated amine, (4) Cl^- permeation, and (5) equilibration of protonated and unprotonated amine.

Theory

Let us assume that tyramine can cross the chromaffin-granule membrane by two pathways: permeation and transport (Figure 1). Unmediated reserpine-insensitive permeation occurs because the unprotonated from of the amine diffuses across the membrane. Translocator-mediated reserpine-sensitive transport occurs by exchange diffusion with H^+. To characterize the steady state created by unmediated amine permeation and translocator-mediated amine transport, we must first describe the kinetics of the two processes individually.

From Fick's law, the rate of unmediated permeation ${}^PV$ is

$$ {}^PV = P(C^0_{out} - C^0_{in}) = P(C^+_{out}K_a/[H^+]_{out} - C^+_{in}K_a/[H^+]_{in}) \quad (1) $$

where P is the permeability coefficient of the unprotonated amine and K_a is the association constant. C^0_{in}, C^0_{out}, C^+_{in}, and C^+_{out} are the internal and external concentrations of the neutral and protonated forms of the amine. Because the pK_a of tyramine is 10.6 (Armstrong & Barlow, 1976), the amine is nearly completely protonated at neutral pH, and we can substitute total internal and external amine concentrations (C_{in} and C_{out}) for C^+_{in} and C^+_{out}. Permeation will reach equilibrium when the concentration of unprotonated amine is the same on both sides of the membrane ($C^0_{out} = {}^0_{in}$). The total internal amine concentration at permeation equilibrium (${}^PC_{in}$) is

$$ {}^PC_{in} = C_{out}[H^+]_{in}/[H^+]_{out} \quad (2) $$

Therefore, eq 1 can be written as

$$ {}^PV = (PK_aC_{out}/[H^+]_{out})(1 - C_{in}/{}^PC_{in}) \quad (3) $$

[1] Abbreviation: Hepes, 2-[N-(2-hydroxyethyl)piperazin-N'-yl]-ethanesulfonic acid.

Table I: Kinetic Parameters for Amine Permeation[a]

conditions	tyramine				dopamine
	V_i	K (uptake)	K (efflux)	P (cm/s)	V_i
50 μM amine, 25 °C, pH 7.2	0.35 ± 0.09 (5)	0.14 ± 0.05 (5)	0.27 ± 0.12 (4)	7.7×10^{-5}	0.043 ± 0.013 (7)
50 μM amine, 25 °C, pH 7.6	0.65 ± 0.10 (3)	0.24 ± 0.06 (3)	0.40 ± 0.07 (4)	5.7×10^{-5}	0.042 ± 0.019 (3)
50 μM amine, 35 °C, pH 7.2	0.49 ± 0.07 (2)	0.27 ± 0.09 (3)	0.39 ± 0.04 (4)	10.8×10^{-5}	0.073 ± 0.024 (3)
550 μM amine, 25 °C, pH 7.2	2.84 ± 0.99 (3)	0.23 ± 0.03 (3)	ND	5.7×10^{-5}	0.264 ± 0.173 (3)

[a] V_i values are in nmol/(min·mg of membrane protein). K values are in min^{-1}. For tyramine, V_i and K (uptake) were determined from uptake experiments (Figure 2), and K (efflux) was from efflux experiments (Figure 3). For dopamine, V_i was determined from uptake experiments as shown in Figure 4. The number of experiments averaged to calculate each value (±standard deviation) is given in parentheses. Values for the permeability coefficient (P) were calculated from V_i by using eq 4.

If we let V_i be the initial velocity of permeation ($^PV = V_i$ when $C_{in} = 0$), then

$$V_i = PK_aC_{out}/[H^+]_{out} \quad (4)$$

and

$$^PV = (dC_{in}/dt)V = V_i(1 - C_{in}/^PC_{in}) \quad (5)$$

where V is the internal volume per unit surface area. Equation 5 can be integrated to give

$$C_{in}(t) = {}^PC_{in} + [C_{in}(0) - {}^PC_{in}] \exp[-V_it/(V{}^PC_{in})] \quad (6)$$

To demonstrate that permeation follows these kinetics, we must show that permeation approaches the equilibrium defined in eq 2 as an exponential function of time and that the initial rate of uptake V_i is that given in eq 4.

Electrogenic amine transport is saturable and obeys Michaelis–Menten kinetics (Knoth et al., 1981b). However, if C_{out} is within an order of magnitude of K_m, transport also approaches equilibrium exponentially.

Therefore, the rate of electrogenic transport (^TV) can be expressed as

$$^TV = [V_{max}C_{out}/(K_m + C_{out})](1 - C_{in}/{}^TC_{in}) \quad (7)$$

$^TC_{in}$ is the internal amine concentration at transport equilibrium. Because transport involves an exchange of $2H^+$ for one protonated amine (Knoth et al., 1981a; Phillips & Apps, 1980; Johnson et al., 1981), the equilibrium internal concentration is

$$^TC_{in} = C_{out}([H^+]_{in}/[H^+]_{out})^2 \exp[F(\psi_{in} - \psi_{out})/(RT)] \quad (8)$$

Consequently, transport and permeation approach different equilibria, and $^TC_{in}$ is generally much greater than $^PC_{in}$.

At steady state, a balance is achieved between electrogenic transport and electroneutral permeation:

$$[V_{max}C_{out}/(K_m + C_{out})](1 - C_{in}/{}^TC_{in}) = -V_i(1 - C_{in}/{}^PC_{in}) \quad (9)$$

C_{in} will lie between $^TC_{in}$ and $^PC_{in}$ depending on the relative values of V_{max} and V_i. Equation 9 can be rearranged to express the kinetic parameters for transport as a function of the steady-state concentration gradients:

$$\frac{V_{max}}{K_m + C_{out}} = \frac{(V_i/C_{out})[(C_{in}/C_{out})/({}^PC_{in}/C_{out}) - 1]}{1 - (C_{in}/C_{out})/({}^TC_{in}/C_{out})} \quad (10)$$

Results

Electroneutral tyramine permeation can be observed in isolation by blocking transport using reserpine. Reserpine-insensitive tyramine permeation approaches equilibrium as an exponential function of time for both influx and efflux (Figures 2 and 3). Points were fit to an exponential curve ($y = A + Be^{-Kt}$) by a least-squares method to determine values for the exponential rate constant (K) and initial velocity of permeation

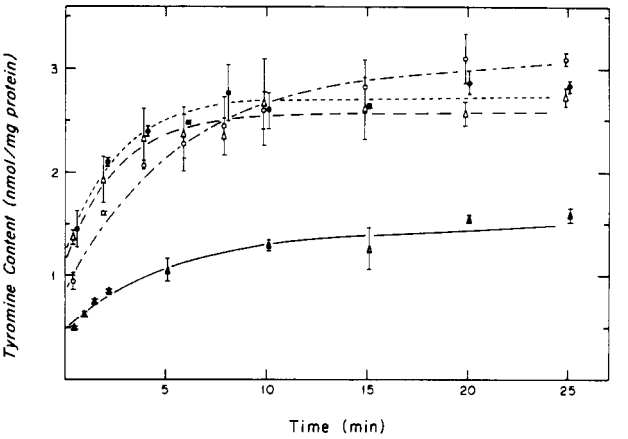

FIGURE 2: Kinetics of reserpine-insensitive tyramine uptake. For each series of points, 0.25 mL of a ghost suspension was mixed with 9.75 mL of 250 mM KCl, 150 mM sucrose, 40 mM Hepes, and 2 μM reserpine and 0.25 mL of 100 mM ATP and 100 mM $MgSO_4$. The suspension was incubated for 20 min at the indicated temperature and pH. Then, uptake was initiated by adding 100 μL of 5 mM [^{3}H]tyramine (50 μCi/mL). One sample (▲) received an additional 50 μL of 0.1 M cold tyramine. At the times indicated, 1-mL samples were assayed for tyramine uptake as described under Materials and Methods. Each point is the average of two replicate samples. Incubation conditions are as follows: (○) pH 7.2, 25 °C, 50 μM tyramine, 3.09 mg of ghost protein; (●) pH 7.6, 25 °C, 50 μM tyramine, 1.93 mg of protein; (△) pH 7.2, 35 °C, 50 μM tyramine, 1.93 mg of protein; (▲) pH 7.2, 25 °C, 550 μM tyramine, 1.85 mg of protein. For the latter sample, plotted values are measured values of the tyramine content divided by 11. Root-mean-square errors for lines fit to the data points are (○ - - -) 0.21, (● - - -) 0.16, (△ - - -) 0.26, and (▲ —) 0.11.

($V_i = BK$). The exponential rate constants for tyramine influx and efflux are comparable (Table I). As predicted by eq 4, V_i is inversely proportional to H^+_{out} and proportional to C_{out}. The permeability coefficient (P) for the unprotonated tyramine species was calculated from V_i by using eq 4 (Table I). The K_a of tyramine was taken to be 2.5×10^{-11} M (Armstrong & Barlow, 1976). The surface area of the chromaffin-granule membrane was estimated to be 3800 cm^2/mg of membrane protein. This was calculated assuming a granule diameter of 285 nm, an internal volume of 4.5 μL/mg of total protein, and a membrane protein:total protein ratio of 0.23 (Njus et al., 1981).

Reserpine-insensitive dopamine influx was too slow to be fitted to an exponential curve (Figure 4). Consequently, V_i values were determined by fitting a straight line to the initial data points. Much of the apparent dopamine uptake is probably caused by binding rather than permeation. If the amine content of the ghosts is measured by collecting the ghosts on membrane filters and washing the filters with distilled water instead of an isotonic solution, the ghosts should lyse, leaving only bound amine on the filter. Under these conditions, tyramine and dopamine both give results comparable to those shown in Figure 4 (data not shown). For tyr-

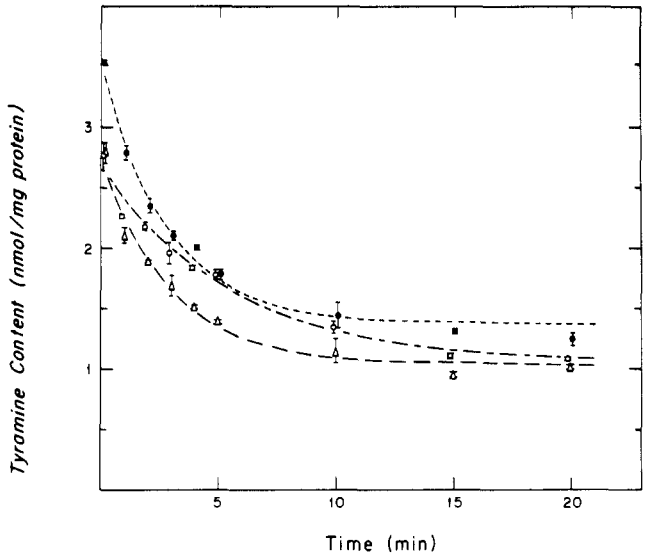

FIGURE 3: Kinetics of reserpine-insensitive tyramine efflux. A ghost suspension (0.25 mL) was incubated with 15 μL of 100 mM ATP and 100 mM $MgSO_4$, 3 μL of 200 μM reserpine, and 50 μL of 5 mM [^{3}H]tyramine at the indicated pH and temperature for 20 min. Then, at $t = 0$, the ghost suspension was added to 9.75 mL of 250 mM KCl, 150 mM sucrose, and 40 mM Hepes at the same pH and temperature. Tyramine efflux was followed by assaying tyramine content as described under Materials and Methods. Each point is the average of two replicate samples. Incubation conditions are the following: (○) pH 7.2, 25 °C, 3.01 mg of ghost protein; (△) pH 7.2, 35 °C, 3.01 mg of protein; (●) pH 7.6, 25 °C, 2.86 mg of protein. Root-mean-square errors for lines fit to the data points are (○---) 0.10, (△--) 0.11 and (●---) 0.10.

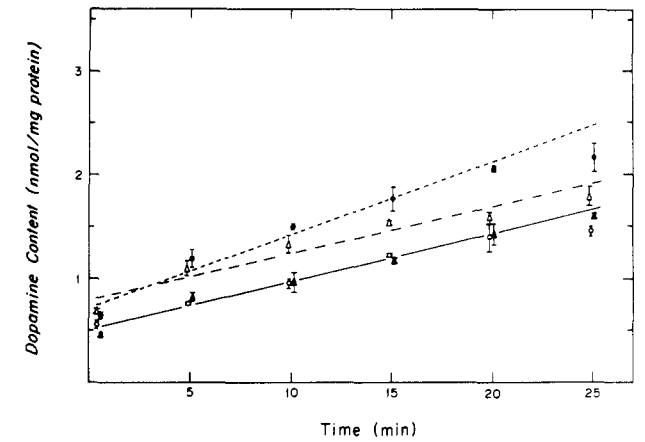

FIGURE 4: Kinetics of reserpine-insensitive dopamine uptake. For each series of points, 0.25 mL of a ghost suspension was mixed with 9.75 mL of 250 mM KCl, 150 mM sucrose, 40 mM Hepes, and 2 μM reserpine and 0.25 mL of 100 mM ATP and 100 mM $MgSO_4$. The suspension was incubated for 20 min at the indicated temperature and pH. Then, uptake was initiated by adding 100 μL of 5 mM (50 μCi/mL) [^{3}H]dopamine (△), 100 μL of 5 mM (25 μCi/mL) [^{14}C]dopamine (○, ●), or 100 μL of 55 mM (25 μCi/mL) [^{14}C]-dopamine (▲). At the times indicated, 1-mL samples were assayed for dopamine uptake as described under Materials and Methods. Each point is the average of two replicate samples. Incubation conditions are as follows: (○) pH 7.2, 25 °C, 50 μM dopamine, 1.00 mg of protein; (△) pH 7.2, 35 °C, 50 μM dopamine, 2.07 mg of protein; (●) pH 7.6, 25 °C, 50 μM dopamine, 1.00 mg of protein; (▲) pH 7.2, 25 °C, 550 μM dopamine, 1.00 mg of protein. For the latter sample, plotted values are measured values of the dopamine content divided by 11. Root-mean-square errors for lines fit to the data points are (○—) 0.07, (△--) 0.11, (●---) 0.11, and (▲—) 0.08.

amine, this binding is small compared to uptake. For dopamine, however, binding seems to be the major component. That permeation of the unprotonated dopamine species is not significant is also indicated by the pH independence of V_i (Table I). Consequently, the rate of dopamine permeation is probably much smaller than the measured V_i values.

Table II: Steady-State Concentration Gradients: 25 °C, pH 7.2, 50 μM Amine[a]

additions	C_{in}/C_{out} tyramine	dopamine	methylamine
ATP	10.5 ± 0.5	51.6 ± 2.5	6.0 ± 0.2
ATP + reserpine	8.8 ± 1.2	11.8 ± 0.6	8.8 ± 0.3
none	3.8 ± 0.3	9.7 ± 2.0	4.4 ± 0.3
reserpine	4.4 ± 0.5	7.6 ± 0.1	4.2 ± 0.0

[a] Each sample contained 2.27 mg of membrane protein. Final concentrations of ATP and reserpine were 5 mM and 2.5 μM, respectively. Gradients were measured after a 60-min incubation. Values are each the average (±SD) of two replicate samples.

Table III: Steady-State Concentration Gradients: 25 °C, pH 7.6, 20 μM Amine[a]

additions	C_{in}/C_{out} tyramine	dopamine	methylamine
ATP	21.7 ± 3.6	193 ± 30	9.8 ± 0.5
ATP + reserpine	11.0 ± 1.7	16.9 ± 3.0	12.3 ± 0.5
none	5.1 ± 0.4	20.3 ± 4.5	5.7 ± 0.1
reserpine	4.6 ± 0.9	13.1 ± 0.3	6.6 ± 0.5

[a] Each sample contained 1.06 mg of membrane protein. Final concentrations of ATP and reserpine were 5 mM and 10 μM, respectively. Gradients were measured after a 60-min incubation. Values are each the average (±SD) of two replicate samples.

Table IV: Steady-State Concentration Gradients: 35 °C, pH 7.2, 20 μM Amine[a]

additions	C_{in}/C_{out} tyramine	dopamine	methylamine
ATP	34.7 ± 10.7	221 ± 36	6.2 ± 1.3
ATP + reserpine	9.2 ± 0.5	12.5 ± 1.2	7.3 ± 0.6
none	3.6 ± 0.2	11.1 ± 4.0	5.0 ± 1.2
reserpine	3.2 ± 0.5	6.7 ± 1.0	4.9 ± 0.2

[a] Each sample contained 0.81 mg of membrane protein. Final concentrations of ATP and reserpine were 5 mM and 10 μM, respectively. Gradients were measured after a 40-min incubation. Values are each the average (±SD) of two replicate samples.

To analyze the balance between transport and permeation, concentration gradients for tyramine, dopamine, and methylamine were measured under steady-state conditions (Tables II–IV). Since dopamine crosses the membrane only via the transport pathway, its concentration gradient (in the absence of reserpine) is that expected for transport alone (eq 8). Methylamine crosses only by permeation in its unprotonated form, so its gradient is that expected for permeation alone (eq 2). In the presence of reserpine, tyramine gradients correlate with methylamine gradients, indicating that tyramine is reaching the equilibrium expected for permeation of the unprotonated form of the amine (eq 2). In the absence of reserpine, the tyramine gradient represents a balance between inward transport and outward permeation and is therefore intermediate between the methylamine and dopamine gradients. To allow the amine gradients to reach a steady state, samples were incubated for 60 min at 25 °C or for 40 min at 35 °C. The ghosts were suspended in a medium containing the permeant Cl^- anion (125 mM KCl) so that the H^+-translocating ATPase would generate a pH gradient instead of a membrane potential (Knoth et al., 1980). That ATP caused the expected change in the pH gradient is confirmed by the ATP-dependent increase in the methylamine gradient, which is equal to the $[H^+]$ gradient (eq 2). A comparison of

Table V: Kinetic Parameters for Dopamine Transport[a]

conditions	K_m (μM)	V_{max} [nmol/(min·mg of protein)]	$K_m(1 + I/K_i)$ (μM)
25 °C, pH 7.2	13.4 ± 2.2	1.09 ± 0.39	67.5 ± 12.7
25 °C, pH 7.6	11.5 ± 1.6	1.59 ± 0.56	104.7 ± 9.0

[a] Kinetic parameters for dopamine transport were determined in the absence and presence of 40 μM tyramine as described under Materials and Methods. Each value is the average (±SD) of three separate experiments.

Table VI: Kinetic Parameters for Tyramine Transport[a]

conditions	$V_{max}/(K_m + C_{out})$ [mL/(min·mg of protein)]	K_i (μM)	V_{max} [nmol/(min·mg of protein)]
50 μM tyramine, 25 °C, pH 7.2	0.00659	9.9	0.39
20 μM tyramine, 25 °C, pH 7.6	0.0178	4.9	0.44
20 μM tyramine, 35 °C, pH 7.2	0.0534	9.9	1.6

[a] $V_{max}/(K_m + C_{out})$ values were calculated from steady-state gradients (Tables II–IV) by using eq 10. K_i values were calculated from Table V. V_{max} was calculated assuming K_i as K_m.

methylamine gradients shows that reserpine increases the pH gradient slightly especially in the presence of ATP.

Values for $V_{max}/(K_m + C_{out})$ calculated from eq 10 are shown in Table VI. C_{in}/C_{out} was taken as the tyramine gradient in the presence of ATP only. Because dopamine is essentially impermeant, its concentration gradient represents the equilibrium gradient for the transport process ($^TC_{in}/C_{out}$). Therefore, $^TC_{in}/C_{out}$ was taken as the dopamine gradient in the presence of ATP. Because reserpine has a slight effect on the pH gradient, $^PC_{in}/C_{out}$ was taken as the methylamine gradient in the presence of ATP rather than as the tyramine gradient in the presence of ATP and reserpine.

To estimate K_m values for tyramine, K_i values for the inhibition of dopamine transport were measured (Tables V and VI). By use of these values for K_m, V_{max} values were calculated from $V_{max}/(K_m + C_{out})$ (Table VI).

Discussion

The monoamine transport system in chromaffin granules has a rather broad specificity, transporting 5-hydroxytryptamine (serotonin) as well as epinephrine, norepinephrine, and dopamine. It has been known for some time, however, that chromaffin granules take up tyramine only poorly. Phillips (1974) found that the V_{max} for tyramine transport is only one-seventh that of (−)-norepinephrine. DaPrada et al. (1975) found that tyramine uptake is only about half that of dopamine and (−)-norepinephrine. They also observed that tyramine uptake is only partially reserpine sensitive whereas dopamine and norepinephrine uptake are completely inhibited by reserpine. Recently, Scherman & Henry (1980) and Johnson et al. (1982) suggested that tyramine is transported by the chromaffin-granule amine translocator but that it is not accumulated because it leaks out of the vesicles. Not knowing the rate of tyramine permeation, however, they could not speculate about the kinetics of tyramine transport.

We have measured rates of tyramine permeation and steady-state levels of tyramine accumulation. From these, we have deduced rates of tyramine transport. To use this approach, we must show that the equations describing permeation and transport are valid. That permeation is described by eq 6 is indicated by the fact that the kinetics of uptake and efflux are exponential (Figures 2 and 3) and the rate constants are the same for both uptake and efflux (Table I). As predicted by eq 4, the rate constant is inversely proportional to the H^+ concentration and is independent of the tyramine concentration (Table I). As required by eq 2, the equilibrium uptake level in the presence of reserpine is close to that for the weak base methylamine (Tables II–IV). Finally, the permeability coefficient calculated for the unprotonated tyramine is comparable to the permeability coefficients of other lipophilic alcohols such as 1-butanol or cyclohexanol measured in the erythrocyte membrane (Naccache & Sha'afi, 1973). These observations are all consistent with permeation of the unprotonated tyramine species. Further evidence for tyramine permeation has been published by Johnson et al. (1982) and Scherman & Henry (1980).

The kinetics of tyramine transport must be modeled in a less straightforward manner. We assume that tyramine transport has the same characteristics as transport of the impermeant substrates dopamine, epinephrine, norepinephrine, and serotonin. For the latter compounds, the initial velocity of transport follows Michaelis–Menten kinetics (Phillips, 1974; Knoth et al., 1981b). Therefore, eq 7 correctly describes the initial velocity [$V_{max}C_{out}/(K_m + C_{out})$]. The equilibrium level of accumulation reached by dopamine transport is that given by eq 8 (Knoth et al., 1981a). Finally, although dopamine transport follows Michaelis–Menten kinetics, the approach to equilibrium is approximately exponential at least for dopamine concentrations within an order of magnitude of the K_m (Knoth et al., 1981a; J. Knoth, J. O. Peabody, and D. Njus, unpublished observations). Therefore, eq 7 adequately describes the kinetics of dopamine transport and by extrapolation tyramine transport as well.

To estimate the K_m for tyramine transport, we measured K_i for the inhibition of dopamine transport (Table V). The values of V_{max} for dopamine transport are comparable to those measured before at 30 °C (Knoth et al., 1981b). Values of K_m are somewhat smaller, but this may reflect improved sealing of the chromaffin-granule ghosts. Without correcting for permeation, Phillips (1974) found the K_m for tyramine transport to be 8 μM. Our measurements of the K_i for inhibition of dopamine transport are comparable. Using the value of K_i for K_m, we find the rate of tyramine transport to be about one-third the rate of dopamine transport. The translocator evidently binds tyramine somewhat more tightly than dopamine but transports it more slowly.

The transport of tyramine has some important implications for the specificity and function of the monoamine translocator. The second hydroxyl group apparently is not required for the binding and transport of the amine. The additional hydroxyl group is important because it renders the amine impermeant and capable of being stored within the storage granules. In this regard, it is interesting to note that tryptamine is also poorly accumulated by chromaffin granules (Da Prada et al., 1975) whereas 5-hydroxytryptamine is taken up efficiently. In this case, too, the additional hydroxyl group may make the molecule impermeant. The hydroxylation enzymes, tyrosine hydroxylase and tryptophan hydroxylase, may have evolved simply to create impermeant and storable transmitter molecules.

Tyramine has some potent physiological effects because it causes depletion of catecholamine stores in vivo. In the presence of monoamine oxidase inhibitors, which prevent the metabolism of tyramine, tyramine ingestion increases pulse

rate and blood pressure and may have more severe effects including headache, nausea, and even death (Horwitz et al., 1964). Von Euler & Lishajko (1968) suggested that tyramine may cause catecholamine release because it is more effective at inhibiting amine uptake than at inhibiting amine efflux. In a study of splenic nerve granules, they found that norepinephrine reuptake was inhibited by about 10 μM tyramine but norepinephrine release was not inhibited until the tyramine concentration was increased to 1 mM. Apparently, at concentrations between 10 μM and 1 mM, tyramine blocks norepinephrine influx but not efflux. Although this unidirectional inhibition of amine transport would not be expected of a typical competitive inhibitor, we can rationalize this behavior in terms of the properties of tyramine discussed above. Since tyramine has a K_i of about 5–10 μM (Table VI), inhibition of catecholamine uptake at these concentrations is expected. Because tyramine leaks out of the vesicles, however, its internal concentration and therefore its effectiveness in inhibiting efflux are reduced. The external tyramine concentration must be raised considerably above the concentration required to inhibit influx before a comparably inhibitory tyramine-to-norepinephrine ratio is achieved on the inside. It should be noted that this unidirectional inhibition of transport does not violate the second law of thermodynamics because it is not achieved without cost. It is supported by the energy expended by the granules in their futile effort to accumulate tyramine. This unusual effect, competitive inhibition of uptake without inhibition of efflux, may account for the amine-depleting effect of tyramine.

The approach used here to measure kinetic parameters for tyramine transport may be generally applicable to study the transport of moderately permeant substrates of other systems. The substrate must be slowly permeant so that it is taken up to a steady-state level above permeation equilibrium. The rate of permeation must be measurable independently of transport, and an impermeant substrate must be available for comparison.

Registry No. Tyramine, 51-67-2; dopamine, 51-61-6.

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