Mechanism of Nasal Absorption of Drugs II: Absorption of L-Tyrosine and the Effect of Structural Modification on its Absorption

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Abstract □ The nasal absorption of L-tyrosine and the effect of structural modification on that absorption have been studied using an in-situ experimental technique. The extent of nasal absorption of the amino acid was found to be the same at pH values of 4.0 and 7.4 but dependent on concentration in the range of 2.8×10^{-4}–2.2×10^{-3} M. *O*-Acyl-L-tyrosine esters, although possessing higher octanol-water (pH 7.4) partition coefficients, have the same rate of nasal absorption as the parent amino acid. *N*-Acetyl-L-tyrosine, on the other hand, was found to have both partition coefficient and nasal absorption rate similar to those of L-tyrosine. Esterification of the carboxyl moiety of L-tyrosine results in derivatives that hydrolyze in the in-situ perfusion medium generating the original amino acid. The rate of nasal absorption of these derivatives was, therefore, determined from an overall disappearance rate which accounted for the rate of hydrolysis to L-tyrosine. These carboxylic esters were absorbed 4 to 10 times faster than L-tyrosine. Although the carboxylic esters of L-tyrosine possess higher octanol-water partition coefficients than the parent amino acid, the differences in the rates of nasal absorption could not be attributed solely to partition coefficient. The enhancement in the rate of absorption observed for these esters was attributed instead to the absence of the negative charge on the carboxylate moiety. It is a result of this negative charge that the rates of nasal absorption of L-tyrosine, *O*-acyl-L-tyrosine esters and *N*-acetyl-L-tyrosine are similar, despite significant differences in their partition coefficients.

Nasal administration of certain lipophilic drugs such as propranolol,[1–3] progesterone,[4] 17β-estradiol,[5] naloxone[6] and testosterone[7] has been shown to result in drug-blood levels similar to those observed following intravenous administration. These drugs undergo extensive metabolism in the gastrointestinal tract and liver following oral administration, yet their nasal administration resulted in rapid and complete absorption. The nasal bioavailability of propranolol in humans, dogs and rats, for example, was found to be 100% whereas its oral bioavailability was only 25, 6.8 and 19%, respectively.

Peptides, on the other hand, have poor nasal bioavailability.[8–12] Although the mechanism of nasal absorption of peptides is not fully understood,[13] it seems reasonable that among the factors contributing to their poor bioavailability might be their polarity. Therefore, the preparation of more lipophilic peptide prodrugs might result in enhanced nasal absorption. Since peptides are amino acid derivatives, the nasal absorption of a model amino acid, L-tyrosine, as well as the effect of structural modification on that absorption were studied. Since any of the various polar functional groups of L-tyrosine could influence its nasal absorption, a stepwise approach designed to isolate the effect of each group was followed. In order to determine the influence of the hydroxyl group on nasal absorption, the *O*-acyl esters were prepared and studied. The influence of the amino group was examined using *N*-acetyl-L-tyrosine and that of the carboxyl group by studying the nasal absorption of the methyl-, ethyl-, *n*-propyl-, and *tert*-butyl carboxylic acid esters. The knowledge acquired from these studies should provide additional understanding of the mechanism of nasal absorption of peptides.

$$ROC_6H_4CH_2CH(NHR^2)CO_2R'$$

1a, $R = R^1 = R^2 = H$
1b, $R = R^2 = H$; $R^1 = CH_3$
1c, $R = R^2 = H$; $R^1 = C_2H_5$
1d, $R = R^1 = H$; $R^2 = CH_3CO$
1e, $R = R^2 = H$; $R^1 = C_4H_9$
1f, $R = CH_3CO$; $R^1 = R^2 = H$
1g, $R = C_4H_9CO$; $R^1 = R^2 = H$
1h, $R = C_5H_{11}CO$; $R^1 = R^2 = H$
1i, $R = R^2 = H$; $R^1 = C_3H_7$

Results and Discussion

The in-situ perfusion studies show that the extent of nasal absorption of L-tyrosine after 60 minutes was the same (~13%) at pH values of 4 and 7.4. On the other hand, the nasal absorption of the amino acid was found to be concentration dependent in the range of 2.8×10^{-4} M to 2.22×10^{-3} M. If the mechanism of nasal absorption follows the Michaelis-Menten process, eq. 1 is valid:

$$\frac{1}{\text{Initial Rate}} = \frac{k_M}{V_{max}S} + \frac{1}{V_{max}} \quad (1)$$

and a plot of the reciprocal of the initial rate for the disappearance of L-tyrosine versus the reciprocal of the initial concentration of the amino acid would result in a straight line. Such a plot is shown in Fig. 1. From the slope and intercept of the line, k_M and V_{max} were calculated to be 4.8×10^{-4} M and 3.39×10^{-4} M h^{-1}, respectively. These data indicate that L-tyrosine is absorbed from the nasal cavity in its zwitterionic form and that its absorption is probably a carrier-mediated process.

As shown in Table I, the apparent rates of nasal absorption of the *O*-acyl-L-tyrosine esters are not significantly different from that of L-tyrosine despite the fact that their octanol-water partition coefficients are considerably greater. The fact that L-tyrosine and its more lipophilic *O*-acyl-derivatives exhibit the same rate of nasal absorption may be attributed to the similarity in the ionic character of the molecules. At the pH values studied, both the parent amino acid and its *O*-acyl esters exist in the zwitterionic state.

The significance of ionic character was elaborated further by the studies employing *N*-acetyl-L-tyrosine and the carboxylic acid esters of the amino acid. Acetylation of the amino

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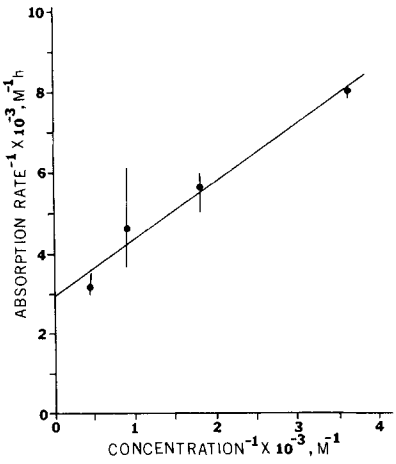

Figure 1—*Lineweaver-Burk plot of the in-situ nasal absorption of L-tyrosine at pH 7.4 and 37°C. Values are mean ± SEM (n = 4–5).*

Table I—Apparent Partition Coefficients, Overall Apparent Nasal Absorption Rate Constants (k_{obs}), Calculated Nasal Absorption Rate Constants (k_a), and Apparent Rate Constants of L-Tyrosine Formation (k_{hyd})

Compound	Apparent Partition Coefficient	k_{obs}, min^{-1} (Mean ± SEM)[a]	k_a, min^{-1}	k_{hyd}, min^{-1}
1a	0.026	0.0023 ± 0.00037	—	—
1b	1.97	—	0.012	0.00243
1c	5.20	—	0.025	0.00357
1d	20.79	—	0.023	0.00415
1e	62.50	—	0.011	0.00084
1f	0.047	0.002 ± 0.00044	—	—
1g	1.170	0.002 ± 0.00017	—	—

[a] n = 4 rats.

group did not significantly change the partition coefficient or enhance the rate of nasal absorption. After a one hour perfusion, only ~6% of the *N*-acetyl-L-tyrosine was absorbed while ~15% of the parent L-tyrosine was absorbed in a similar time period.

Esterification of the carboxylic acid functional group of L-tyrosine, however, results in derivatives that exhibit both higher partition coefficients and more rapid absorption rates than the parent amino acid. As shown in Table I, the partition coefficients for the methyl-, ethyl-, *n*-propyl- and *tert* esters increase from about 100 to more than 2400 times that of L-tyrosine. The carboxylic acid esters were found to hydrolyze during the in-situ perfusion study generating L-tyrosine. The apparent pseudo first-order rate constants for these hydrolyses (k_{hyd}) are also shown in Table I.

During the nasal absorption studies on these compounds, therefore, hydrolysis as well as absorption contributed to the overall disappearance of carboxylic acid ester from the perfusion medium. Since an HPLC method of analysis was used to monitor the concentration of all the species involved, it was possible to determine the contribution of the individual processes to the overall rate using eqs. 2 and 3.

$$-\frac{dC}{dt} = k_a[C] + k_{hyd}[C] \quad (2)$$

$$k_{obs} = k_a + k_{hyd} \quad (3)$$

where C is the concentration of ester, k_{obs} is the overall apparent first-order nasal absorption rate of the ester, k_a is the calculated first-order rate constant of ester absorption and k_{hyd} is the rate constant of hydrolysis described above. This method of calculating the contribution of k_{hyd} to the overall apparent nasal absorption rate of ester treats the absorption of the parent amino acid as a first-order process. Although an active-transport absorption constant might have been employed, such a treatment was not critical to the calculation of individual rate constants for the following reasons. The overall rate of disappearance of the prodrug observed was an order of magnitude faster than the rate of formation of L-tyrosine and, the extent of absorption of L-tyrosine is extremely small.

It is apparent that the rates of absorption of the carboxylic acid esters of L-tyrosine are about 4 to 10 times faster than that of L-tyrosine itself. Figure 2 is a typical plot showing the disappearance of the *n*-propyl ester and the appearance of L-tyrosine during an in-situ perfusion.

Although the 1-octanol-water partition coefficients of the carboxylic acid esters of L-tyrosine were found to be many fold greater than that of L-tyrosine (Table I), the enhancement in absorption rate observed for these esters cannot be rationalized solely on differences in partition coefficient between the derivatives and *L*-tyrosine. For example, the *O*-valeryl-L-tyrosine exhibited a partition coefficient of approximately the same value as that of the methyl ester of *L*-tyrosine (1.17 versus 1.97), yet there was a four-fold difference in their rates of absorption (0.0029 versus 0.012 min^{-1}).

Based on the results above, it may be concluded that the enhancement in the nasal absorption of the carboxylic acid esters of L-tyrosine is the result of the masking of the negative charge on the carboxylate moiety. It is a result of this negative charge that L-tyrosine, *O*-actyl-L-tyrosine and *N*-acetyl-L-tyrosine have similar rates of nasal absorption, despite the significant differences in their partition coefficient.

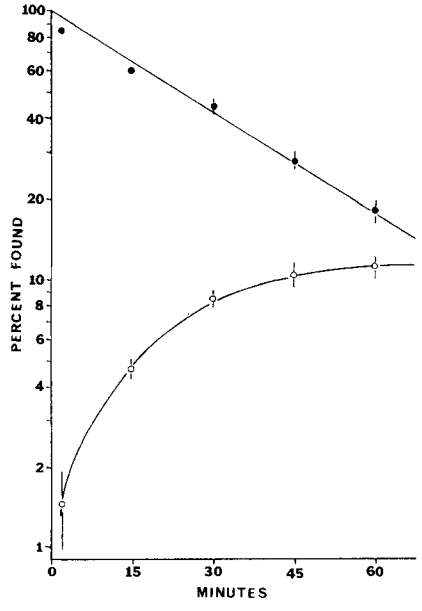

Figure 2—*Disappearance of L-tyrosine n-propyl ester and appearance of L-tyrosine in the in-situ perfusing solution as a function of time at pH 7.4 and 37°C. Key: (●) L-tyrosine n-propyl ester; (○) L-tyrosine. Values are mean percent ± SEM (n = 4).*

Conclusions

L-tyrosine was found to absorbed nasally via a carrier-mediated process which is independent of pH at pH values of 4.0 and 7.4. Acylation of the amino group on the L-tyrosine molecule had no effect on the lipophilicity or rate of nasal absorption of the amino acid. On the other hand, esterification of the phenolic and carboxyl groups result in derivatives considerably more lipophilic than L-tyrosine. The in-situ rates of nasal absorption of the carboxylic acid esters were significantly greater than that of L-tyrosine, whereas the *O*-acyl esters were absorbed with rates similar to that of the amino acid. It is concluded that the enhancement of the rate of nasal absorption observed for the L-tyrosine carboxylic acid esters is due to the masking of the negative charge on the carboxylate moiety.

Experimental Section

Materials—L-tyrosine (**1a**), L-tyrosine methyl ester (**1b**), L-tyrosine ethyl ester (**1c**) and *N*-acetyl-L-tyrosine (**1d**) (Sigma Chemical Company, St. Louis, MO) and L-tyrosine *tert*-butyl ester (**1e**) (Accurate Chemical and Scientific Corporation, Westbury, NY) were used as purchased. *O*-Acetyl-L-tyrosine (**1f**), *O*-valeryl-L-tyrosine (**1g**) and *O*-hexanoyl-L-tyrosine (**1h**) were synthesized according to a procedure utilized previously in these laboratories for epinephrine esters.[14] L-Tyrosine-*n*-propyl ester (**1i**) was synthesized according to the method of Filho and Goissis.[15]

Chemistry—The *O*-acyl-L-tyrosine esters (**1f**, **1g** and **1h**) were prepared by the dropwise addition of an excess of the appropriate acid chloride to a solution of 2 g (11.0 mmol) of L-tyrosine and 1.75 mL of perchloric acid (70%) in 30 mL of ethyl acetate. The resulting mixtures were slowly warmed to reflux and after 5 hours cooled to room temperature. The cooled mixtures were then concentrated under reduced pressure to ~5 mL and dissolved in 100 mL of anhydrous ether. The crude products were precipitated by the addition, with stirring, of 200 mL of water and sufficient ammonium hydroxide to maintain a neutral pH and were recovered by filtration.

Crude **1f** was recrystallized from methanol to afford a 70% yield of a white powder, mp 198–199°C; ^{1}H NMR(D_2O): δ 2.2 (s, 3, CH_3CO), 3.2–3.4 (d, 2, $ArCH_2$), 4.4–4.6 (t, 1, $CH_2CH(NH_2)$), 6.8–7.2 ppm (m, 4, ArH).

Anal.—Calc. for $C_{11}H_{13}NO_4$: C, 59.2; H, 5.87; N, 6.28. Found: C, 59.2; H, 6.03; N, 6.19.

Crude **1g** was washed with ether and recrystallized from methanol to afford a 60% yield of a white powder, mp 189–190°C; ^{1}H NMR (CD_3OD: 38% DCl 9:1): δ 0.9–1.0 (m, 3, CH_3-), δ 1.3–1.5 (m, 2, CH_3CH_2), 1.6–1.8 (m, 2, $C_2H_5CH_2$), 2.5–2.7 (t, 2, $C_3H_7CH_2CO$), 3.1–3.2 (m, 2, $ArCH_2$), 4.2–4.3 (t, 1, $ArCH_2CH(NH_2)$), 6.8–7.2 ppm (m, 4, ArH).

Anal.—Calc. for $C_{14}H_{19}NO_4$: C, 63.4; H, 7.2; N, 5.28. Found: C, 63.25; H, 7.14; N, 5.17.

Crude **1h** was washed with ether and recrystallized from methanol to afford a 65% yield of a white powder, mp 193.5–194.0°C; ^{1}H NMR (CD_3OD: 38% DCl 9:1): δ 0.9 (t, 3, CH_3), δ 1.3–1.7 (m, 6, $CH_3(CH_3)$), 2.5–2.7 (t, 2, $C_4H_9CH_2CO$), 3.2–3.4 (m, 2, $ArCH_2$), 4.3–4.4 (m, 1, $ArCH_2CH(NH_2)$), 6.8–7.5 ppm (m, 4, ArH).

Anal.—Calc. for $C_{15}H_{21}NO_4$: C, 64.49; H, 7.5; N, 5.0. Found: C, 63.93; H, 6.91; N, 4.66.

Compound **1i** was prepared by the dropwise addition, with stirring, of 10 mL of thionyl chloride to 40 mL of *n*-propyl alcohol at −5°C. After 20 min the mixture was allowed to warm to room temperature and treated in three aliquots with a slurry of 2.3 g (12.7 mmol) L-tyrosine in 10 mL of *n*-propyl alcohol. The resulting mixture was then refluxed (~70°C) for 2 h and cooled to room temperature. Crude product was precipitated as the hydrochloride salt by the addition at dry-ice temperature of anhydrous ether. The precipitate was recovered by filtration, washed with anhydrous ether and recrystallized from methanol:ether (10:1) to afford a 76% yield of a white powder, mp 158.0–158.5°C; ^{1}H NMR (CD_3OD): δ 0.8–1.1 (t, 3, CH_3), 1.4–1.9 (m, 2, CH_3CH_2), 3.1–3.3 (d, 2, $ArCH_2$), 4.0–4.2 (m, 2, $COOCH_2$), 4.3–4.4 (m, 1, $ArCH_2CH(NH_2)$), 6.8–7.3 ppm (m, 4, ArH).

Anal.—Calc. for $C_{12}H_{18}ClNO_3$: C, 55.5; H, 6.98; N, 5.39. Found: C, 55.7; H, 7.22; N, 5.25.

Equipment—Spectrophotometric analyses were performed on a Cary 118 (Varian Associates, Inc., Palo Alto, CA). HPLC analyses were performed using a reversed-phase liquid chromatography system. The system consisted of a solvent delivery pump (Model 6000A; Waters Associates, Inc., Milford, MA), a loop (100 μL) injector (Model 7125; Rheodyne Inc., Cotah, CA), a variable-wavelength detector (Vari-chrom; Varian Associates, Inc., Palo Alto, CA), a recorder (Omni-Scribe Model B-5117-5I; Houston Instruments, Division of Bausch and Lomb, Austin, TX), and a 250 × 4.6 mm 5-μm octyl column (Ultrasphere-Octyl; Altex Scientific, Berkeley, CA). A polystaltic pump (Buchler Instruments, Inc., Fort Lee, NJ) was used to circulate the various perfusates in the in-situ system.

Chromatographic Conditions—Two mobile phases, consisting of 70 parts of 0.02 M citric acid at pH 2.4 and 30 parts of methanol with 0.9% sodium perchlorate (A) or 90 parts of 0.1 M phosphate buffer at pH 6.4 and 10 parts of methanol (B), were used. The flow rate was 1.0 mL/min in each case. The analytical wavelengths, mobile phases and approximate retention times for each compound are: **1a**, 274(A), 3.8 min; **1b**, 278(A), 5.6 min; **1c**, 278(A), 8.0 min; **1d**, 280(B), 5.2 min; **1e**, 278(A), 30.0 min; **1f** 266(A), 7.0 min; **1g**, 221(A), 88.8 min; **1i**, 278(A), 20.0 min.

Nasal Absorption Studies—The nasal absorption of L-tyrosine and its derivatives was studied using an in-situ experimental technique described previously.[13,16] Male, Sprague-Dawley rats, weighing approximately 300 g each, were used. Each drug solution was circulated through the nasal cavity for a period of one hour during which aliquots were withdrawn and analyzed for drug remaining.

For L-tyrosine, experiments designed to study the effect of pH were conducted on solutions containing 2.2×10^{-3} M of the amino acid in each of 0.2 M acetate buffer at pH 4 and 0.1 M phosphate buffer at pH 7.4 while the effect of concentration was studied using solutions containing 2.8×10^{-4} M, 5.5×10^{-4} M, 1.11×10^{-3} M and 2.22×10^{-3} M L-tyrosine in isotonic phosphate buffer at pH 7.4.

The nasal absorption rates of *O*-acetyl- and *O*-valeryl-L-tyrosine in 0.1 M phosphate buffer and of *N*-acetyl-L-tyrosine and the methyl-, ethyl-, *n*-propyl- and *tert*-butyl esters of L-tyrosine in 0.01 M isotonic phosphate buffer were also studied at 37°C. For each derivative, 5 mL of a 2.2×10^{-3} M solution at pH 7.4 was used for the perfusion.

Hydrolysis of the L-Tyrosine Derivatives in the Perfusion Medium—The stability of solutions containing 2.2×10^{-3} M of the various L-tyrosine derivatives in 0.1 M isotonic phosphate buffer at pH 7.4 was determined at 37°C. In a typical run, 20 mL of each solution was placed in a water jacketed beaker (Radiometer Company, Copenhagen, Denmark) and maintained at 37°C by means of a circulating water bath (Model F4391; Haake Instruments, Inc., Rochelle Park, NJ). With constant stirring, each solution was circulated by means of the polystaltic pump through tygon tubing (Norton Company, Akron, OH) at a flow rate of 2 ml/min. Aliquots were withdrawn periodically, acidified to pH 3 with 0.02 M citric acid and frozen until HPLC analysis. The observed first order rate constants were calculated from the slopes of semilogarithmic plots of the peak height of the compound remaining in the perfusing medium versus time.

Partition Coefficient Determination Between 1-Octanol and pH 7.4 Phosphate Buffer—The apparent partition coefficients of the *O*-acyl-L-tyrosine esters, *N*-acetyl-L-tyrosine and L-tyrosine carboxylic acid esters were determined at 37°C between 1-octanol and isotonic 0.1 M phosphate buffer at pH 7.4.

The phosphate buffer and octanol were presaturated with one another before use to minimize the volume changes due to mutual solubility. An aqueous phase was prepared by dissolving appropriate amounts of the various esters in the phosphate buffer to give a final concentration of 2.2×10^{-3} M. The aqueous phase (10 mL) was mixed with 10 mL of 1-octanol. The mixtures were manually shaken for 2 min followed by mechanical shaking at 37°C for 15 min to 1 h to ensure equilibrium. The two phases were then separated. For the *O*-acyl-L-tyrosine esters, the octanol layer was back-extracted twice with fresh 10 mL portions of 0.01 M HCl. For *N*-acetyl-L-tyrosine, the separated octanol layer was back-extracted three times with 0.001 M NaOH. Following centrifugation (Model TJ-6; Beckman Instruments, Inc., Palo Alto, CA) at 7000 rpm for 7 min, the combined extracts were appropriately diluted with citric acid and assayed by HPLC. For the L-tyrosine carboxylic acid esters, separation of the two phases was followed by centrifugation and analysis of the aqueous phase.

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