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NASAL DELIVERY OF ATENOLOL AND TIMOLOL IN THE RAT AND THE EFFECT OF ABSORPTION ENHANCERS

K. P. Thadikonda+, C. A. Lau-Cam*, V. L. Thadikonda
and V. Theofanopoulos

College of Pharmacy & Allied Health Professions
St. John's University, Jamaica, NY 11439

ABSTRACT

This study has investigated the nasal mucosa as an alternative site for the administration of atenolol and timolol maleate, two antihypertensive agents whose oral administration is subject to either incomplete absorption (atenolol) or significant first-pass effect (timolol). To this end, the intranasal absorption of these drugs was first evaluated in an *in vivo* rat model, with and without the absorption enhancers amastatin and sodium glycocholate, and next compared with those obtained after oral and intravenous dosings. Use of the intranasal route resulted in higher plasma drug levels than by the oral route ($p<0.05$) and in systemic bioavailabilities that compared very favorably with those obtained intravenously (ca. 90% for both drugs). Bioavailability of the title drugs from the nasal mucosa improved upon coadministration with an absorption enhancer (96-98%, atenolol; ca. 99%, timolol).

INTRODUCTION

Atenolol and timolol are two β-adrenoceptor antagonists currently used for the treatment of mild to moderate hypertension. Although effective when given orally, their bioavailability by this route is far from complete. For example, in the case of atenolol only about 50 per cent of an oral dose is absorbed from the gastrointestinal tract, with the remainder appearing unchanged in the feces (1,2). Oral delivery of timolol, on the other hand, provides rapid and nearly complete systemic absorption (> 90 per cent) (3),

*To whom correspondence should be addressed.
+Present address: Ogden BioServices Corp., Rockville, MD

but ensuing plasma levels are only about one-half or less of those observed by the intravenous route due to first-pass elimination effect (3-5).

Several studies have shown the nasal cavity to be a suitable route of administration for achieving blood drug levels equivalent to those obtained upon intravenous dosing (6-8), or for providing effective systemic delivery of drugs that do not attain a satisfactory bioavailability by the oral route because of extensive first-pass hepatic metabolism, instability within the gastrointestinal environment, or inefficient and variable absorption (9-11).

Using atenolol and timolol and an *in vivo* rat model for intranasal drug delivery, the present study was undertaken to: (a) determine the suitability of the nasal mucosa as a site for the administration of β-adrenoceptor blockers that exhibit incomplete oral absorption or appreciable first-pass effect; (b) compare the resulting plasma pharmacokinetic parameters with those that follow oral and intravenous administrations; and (c) compare nasal absorptions in the presence and absence of the adjuvants amastatin and sodium glycocholate.

EXPERIMENTAL

Animals and Materials

All experiments were conducted on male Sprague-Dawley rats (Taconic Farms, Germantown, NY), 250-300 g in weight, acclimated for 7 days, and fasted for at least 14 hr before the experiments. Experimental groups consisted of 5 rats each. All drugs and absorption enhancers used in the study (atenolol, timolol maleate, heparin sodium, urethane, amastatin hydrochloride, and sodium glycocholate) were obtained from Sigma Chemical Co., St. Louis, MO. HPLC grade water and acetonitrile, and reagent grade triethylamine and phosphoric acid were from J.T. Baker, Phillipsburg, NJ.

Treatments

Treatment solutions were prepared freshly in physiological saline: atenolol, 1.5 mg/kg; timolol maleate, 0.2 mg/kg; sodium glycocholate, 1%; amastatin hydrochloride, 0.015%. For treatments with a drug plus enhancer, the drug was dissolved in enhancer solution rather than in plain physiological saline.

Solutions were injected intravenously through the tail vein at a volume of 300 μL/kg. Oral treatments were performed using an oral feeding needle and a volume of 1 mL/kg. Intranasal absorption studies were conducted as described earlier (12), on rats previously anesthetized with an intraperitoneal dose of urethane (1 g/5 mL/kg) 15 min

prior to a drug treatment. Treatment solutions (300 μL/kg) were delivered into the nasal cavity through a piece of PE-20 tubing affixed to a 100 μL microsyringe. The animals were kept lying on their backs and under a heat lamp throughout the experiments.

Blood Samples and Drug Assays

Blood samples were collected from the femoral artery, into heparinized test tubes. Plasma samples were separated by immediate centrifugation at 1800 x g and 4°C for 10 min, and analyzed for their drug contents by the HPLC method of Lennard and Parkin (13) with suitable modifications. Separations were accomplished on a Supelcosil C_{18} (timolol) or Econosphere C_{18} (atenolol), 30 cm x 4.6 mm i.d., 5 μm, chromatographic column protected with an equivalent guard column. The mobile phase was a mixture of water-acetonitrile (87+13) containing 1% of triethylamine, and adjusted to pH 3.0 with H_3PO_4. Detection was at 295 (timolol) or 274 (atenolol) nm.

Analysis of Data

Plasma pharmacokinetic parameters were calculated assuming a one-compartment model. A commercial statistical software package (PROC NOLIN program of PC SAS/STAT, SAS Institute Inc., Cary, NC) was used for this purpose. Results are expressed as the mean ± SEM of five determinations. Intergroup differences were established by one-way analysis of variance (ANOVA) and post-hoc tests (Duncan's multiple-test range and Dunnett's tests). Data was also analyzed by Student's t-test. Differences were considered significant at $p<0.05$.

RESULTS

The time courses of the mean plasma levels of atenolol and timolol for the various treatments and routes of administration are shown in Figures 1 and 2, respectively. The pharmacokinetic data derived from these plasma levels are summarized in Tables 1 and 2. Bioavailability results are presented in Figure 3.

In the case of atenolol, the fraction of drug absorbed after oral dosing was only 45% of that by the intravenous route (AUC_{po} 403.13 μg/mL min vs. AUC_{iv} 894.79 μg/mL min). Intranasal delivery of the same dose resulted in a 122% increase in drug absorption and in a much greater absolute bioavailability compared to the oral route (AUC_{in} 803.93 μg/mL min, F = 90%) (Table 1). The mean maximal plasma concentration of atenolol by the intranasal route was achieved in less than 8 min, which was about 20 times faster than after oral dosing. The maximum plasma drug concentration after

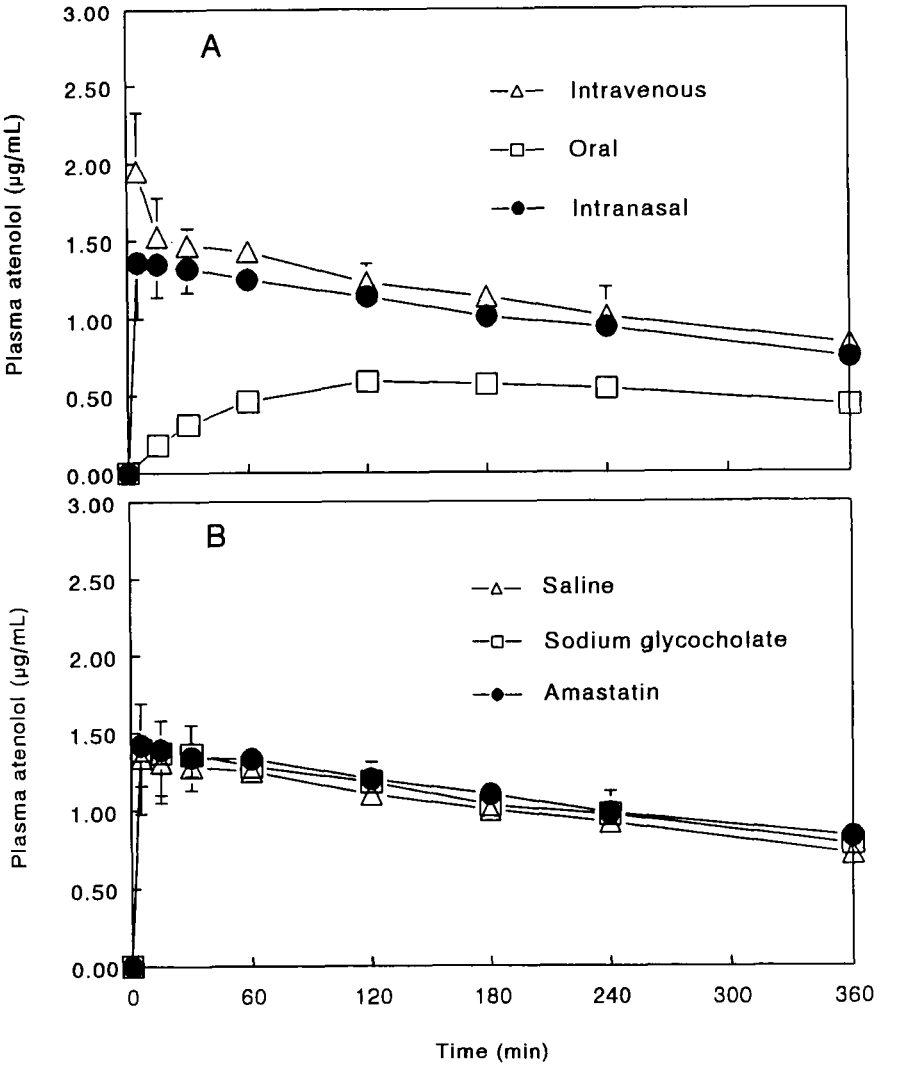

Figure 1. Temporal changes in plasma atenolol levels after the administration of a 1.5 mg/kg dose of atenolol to rats (A) by intravenous, intranasal and oral routes, (B) by intranasal route with and without an adjuvant. Each point represents the mean for 5 rats. Vertical lines represent SEM.

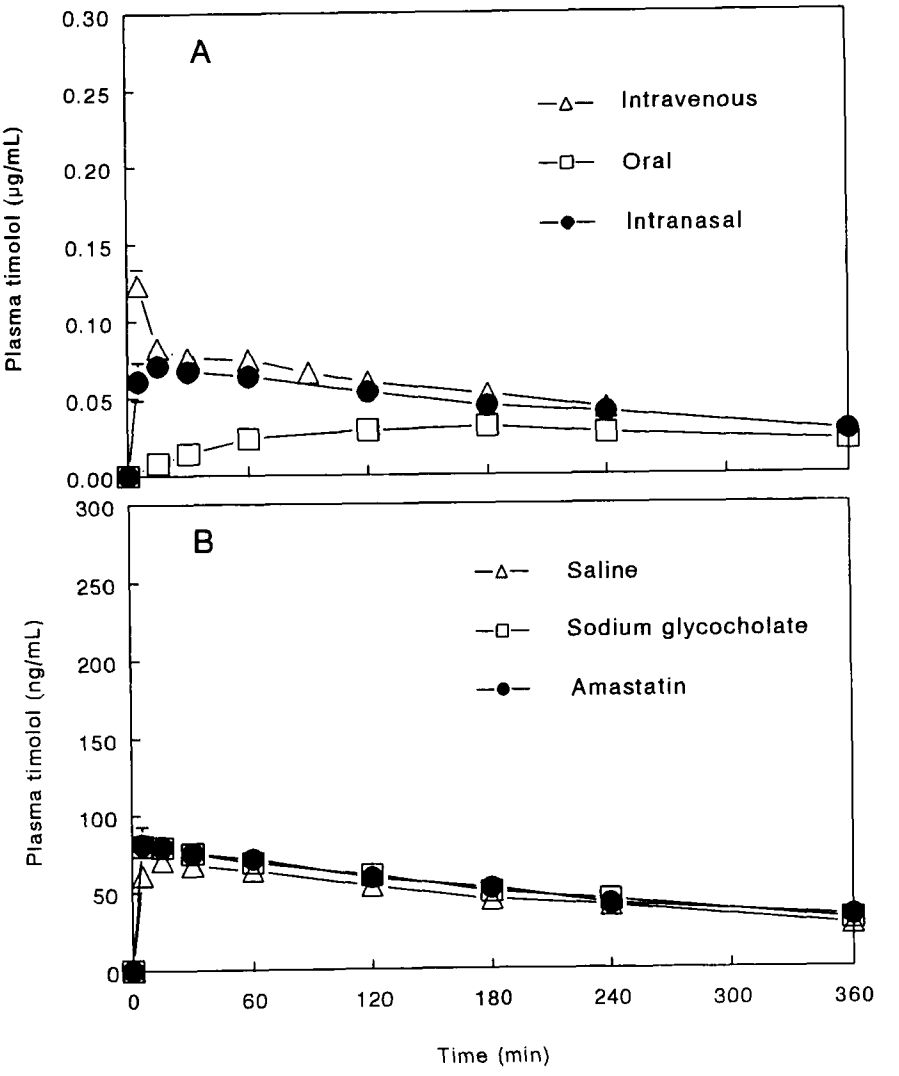

Figure 2. Temporal changes in plasma timolol levels after the administration of a 0.2 mg/kg dose of timolol to rats (A) by intravenous, intranasal and oral routes, (B) by intranasal route with and without an adjuvant. Each point represents the mean for 5 rats. Vertical lines represent SEM.

Table 1

Plasma pharmacokinetic data for atenolol (1.5 mg/kg) after intravenous, oral and intranasal (with and without an absorption enhancer) deliveries[a-c]

Route	K_a (min^{-1})	K_e (min^{-1})	$t_{1/2}$ (hr)	t_{max} (min)	C_{max} (μg/mL min)	AUC (μg/mL min)
Intra-venous	--	0.0017 ±0.0001	6.64 ±0.26	--	--	894.79 ±49.51
Oral	0.0176 ±0.0008	0.0019 ±0.0001	6.01 ±0.44	141.32 ±12.46	0.590 ±0.039	403.13 ±26.28
Nasal	0.81409[d] ±0.1248	0.0017 ±0.0002	6.72 ±0.79	7.58[d] ±1.29	1.370[d] ±0.118	806.93 ±7.92
Nasal + SG	0.9111 ±0.2133	0.0017 ±0.0001	7.00 ±0.58	6.94[d] ±0.99	1.399[d] ±0.269	857.40[d,e] ±41.39
Nasal + AMA	3.5346[f] ±0.3192	0.0016 ±0.0002	7.13 ±0.93	2.18[d,f] ±0.35	1.418[d] ±0.213	878.40[d,e] ±41.39

[a]SG = sodium glycocholate, AMA = amastatin.

[b]Intravenous V_d = 0.97 ± 0.27 L/kg; C_o = 1.547 ± 0.217 μg/mL.

[c]Comparisons were made using ANOVA and Duncan's multiple range test.

[d]Comparisons vs. oral ($p<0.05$).

[e]Comparisons vs. nasal ($p<0.05$).

[f]Comparisons vs. nasal + SG ($p<0.05$).

intranasal delivery was more than twice that seen after oral delivery of the same dose. The intranasal coadministration of atenolol with either amastatin or sodium glycocholate led to an even greater bioavailability than with atenolol alone (F = 98% with amastatin, 96% with sodium glycocholate; Figure 3A). The rate of drug absorption and mean maximal plasma drug concentration in the presence of amastatin were about 4 faster and 3.5 times greater, respectively, than in its absence.

For timolol, the fraction of drug absorbed after oral administration was only 56% of that obtained by the intravenous route (AUC_{po} 17.49 ng/mL min vs. AUC_{iv} 31.21 ng/mL min). Intranasal administration of the same dose of timolol resulted in a systemic

Table 2

Plasma pharmacokinetic data for timolol (0.2 mg/kg) after intravenous, oral and intranasal (with and without an absorption enhancer) deliveries[a-c]

Route	K_a (min^{-1})	K_e (min^{-1})	$t_{1/2}$ (hr)	t_{max} (min)	C_{max} (μg/mL min)	AUC (μg/mL min)
Intra-venous	--	0.0028 ±0.0002	4.14 ±0.30	--	--	31.21 ±2.69
Oral	0.0137 ±0.0018	0.0026 ±0.0004	4.49 ±0.69	150.36 ±8.39	0.031 ±0.004	17.49 ±2.41
Nasal	0.3660[d] ±0.0197	0.0026 ±0.0001	4.38 ±0.17	13.58[d] ±1.46	0.070[d] ±0.029	27.66[d] ±3.12
Nasal + SG	0.9447[e] ±0.2713	0.0026 ±0.0001	4.36 ±0.10	6.24[d] ±1.03	0.080[d] ±0.024	30.77[d,e] ±3.41
Nasal + AMA	1.1428[e] ±0.2489	0.0027 ±0.0001	4.36 ±0.16	5.32[d,e] ±0.99	0.081[d] ±0.018	31.00[d,e] ±2.17

[a]SG = sodium glycocholate, AMA = amastatin.

[b]Intravenous V_d = 2.36 ± 0.33 L/kg; C_o = 0.085 ± 0.009 μg/mL.

[c]Comparisons were made using ANOVA and Duncan's multiple range test.

[d]Comparisons vs. oral ($p<0.05$).

[e]Comparisons vs. nasal ($p<0.05$).

[f]Comparisons vs. nasal + SG ($p<0.05$).

absorption that was about 58% higher than that obtained by the oral route (AUC_{in} 27.66 ng/mL min), and in a greater absolute bioavailability (F = 89% vs. 56%) (Figure 3B). The maximal plasma drug concentration by this route was reached in less than 14 min, which is 10 times faster than by the oral route. As noted with atenolol, the intranasal coadministration of timolol with either amastatin or sodium glycocholate resulted in a greater bioavailability than with timolol alone (F = 99% with both enhancers; Figure 3B). Relative to the nasal administration of the drug alone, the addition of an absorption enhancer reduced the time for maximal plasma concentration by about one-half and accelerated the rate of drug absorption by a factor of 3. Plasma half-lives were not significantly different among the various routes of administration used.

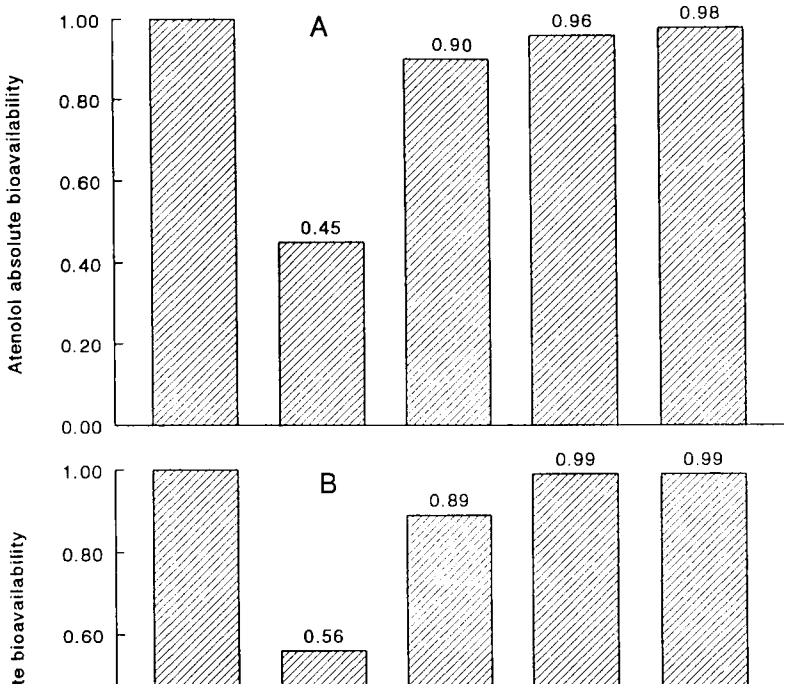

Figure 3. Comparison of absolute bioavailabilities after oral and intranasal (with and without an adjuvant) administrations of (A) atenolol, 1.5 mg/kg, and (B) timolol, 0.2 mg/kg to rats. Key: SG = sodium glycocholate; AMA = amastatin. Values represent the mean for 5 rats.

DISCUSSION

Previous work on the intranasal delivery of alprenolol and metoprolol, two water-soluble β-adrenoceptor blocking agents with similar pK_a values (9.5) and molecular sizes (249.34 and 267.38, respectively) but differing hydrophobicities (based on the apparent octanol/pH 7.4 buffer partition coefficient by the shake method, K'_{SF}), has indicated that hydrophobicity is an important determinant of the rate and extent of nasal drug absorption (14). Indeed, whereas the more hydrophobic alprenolol (K'_{SF} = 9.5) was absorbed

more rapidly and demonstrated a much greater bioavailability by the intranasal route than by the oral route, the more hydrophilic metoprolol (K'_{SF} = 0.5) was hardly absorbed from the nasal mucosa (14). Furthermore, systemic bioavailability of the more hydrophobic β-adrenoceptor antagonist propranolol (K'_{SF} = 13.5) after intranasal delivery has been found to be equivalent to that achieved by the intravenous route (6-8). Similar correlation between hydrophobicity and permeation across biological membranes has also been demonstrated for the buccal administration of atenolol and propranolol (15,16). Like alprenolol and metoprolol, atenolol and timolol also differ in hydrophobicity (K'_{SF} = 0.02 vs. K'_{SF} = 0.59) (17) but, unlike their congeners, they also differ in molecular size (266.34 vs. 361.42) and pK_a value (9.55 vs. 8.80) (17). Bioavailabilities of atenolol and timolol by the oral route were about one-half of those obtained by the parenteral route. However, delivery of these drugs from the nasal cavity led to bioavailabilities that approximated those achieved parenterally and which were not significantly different from each other. These results are rather unexpected since atenolol is less hydrophobic than metoprolol despite their similarities in molecular size, pK_a value, and structural features; and timolol is as hydrophobic as metoprolol, even though its structure contains a 4-morpholino-1,2,5-thiadiazole substituent in place of the smaller and characteristic p-substituted phenyl group of most β-adrenoceptor blocking drugs. The absorption of atenolol through the human buccal membrane was negligible (<2%) at physiological pH, and undetectable at pH over 10. In contrast, the buccal absorption of the more lipophilic compound propranol is found to be about 42% at physiological pH, and to increase with increasing pH (15). These results and those gathered in the present study support the view that large differences between the K'_{SF} values of two β-adrenoceptor blockers may not necessarily translate into marked differences in the rate and extent of their nasal absorption, and that the mechanism of absorption through the nasal membrane may be different from that occurring across other biological membranes (18).

Bile salts are the most frequently studied promoters of nasal absorption (19,20), and sodium glycocholate has been one of the most extensively employed bile salts in *in vivo* (21-23) and *in vitro* (24,25) work. Evaluation of the effects of bile salts on the nasal absorption of β-adrenoceptor blockers appear to be limited to the transport of propranolol through sheep nasal mucosa in an *in vitro* model system (25). In the present study, sodium glycocholate was used in a concentration known to enhance transmucosal absorption while producing minimal irritation and morphological alterations in the nasal mucosa (19,23,26-29), and no apparent ciliotoxic or inhibition of the mucociliary transport rate (26). In comparison to the nasal administration of atenolol and timolol without an adju-

vant, the addition of 1% sodium glycocholate produced a significant increase in the fraction of drug absorbed and, thereby, a greater systemic bioavailability from the nasal mucosa. Although the manner by which sodium glycocholate exerts its absorption enhancing effects is not clearly defined, it is known to vary from drug to drug and to encompass, in some instances, a combination of different mechanisms (21,29).

Based on the proposal that sodium glycocholate may increase nasal membrane permeability by inhibiting the activity of proteolytic enzymes at the absorption site (27), it was also considered of interest to determine if substances possessing this type of effect could influence the nasal absorption of two nonprotein molecules such as atenolol and timolol. The use of an enzyme inhibitor as an absorption enhancer of nonprotein molecules appears not to have been previously reported. In this study, amastatin, an inhibitor of both leucine aminopeptidase and aminopeptidase A (30), was found to be an effective absorption enhancer, surprisingly more so than sodium glycocholate. In the presence of amastatin the rate of drug absorption was faster, the time to maximum plasma drug concentration was shorter, and the systemic bioavailability exceeded 97%, relative to the nasal administration of atenolol or timolol without an adjuvant. The nonprotein nature of the title drugs rules out peptidase activity inhibition as a contributing factor to the noted improvements in nasal absorption. Rather, an excess of peptidase inhibitor might provide a more favorable environment for drug absorption by bringing about either a disturbance of the natural dynamic equilibrium among proteases, peroxidases, and natural protease inhibitors (30, 31), or changes in the conformation of enzyme proteins that represent a barrier within the nasal mucosa (31). Confirmation of any of these mechanisms awaits further investigation.

Summarizing, the present study finds that the administration of a β-adrenoceptor blocking drug of the 2-propanolamine type from the nasal cavity can result in both a faster systemic drug absorption and a greater bioavailability than that achieved by the oral route. Moreover, it also shows that the intranasal coadministration of this type of drug with a bile salt or a peptidase inhibitor can result in a greater systemic bioavailability than by giving the drug alone. Consequently, the nasal route may represent a viable alternative to the oral delivery of β-adrenoceptor blockers whose gastrointestinal absorption may be incomplete, variable, or subject to extensive first-pass effect.

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