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Review

Drug Metabolism in the Nasal Mucosa

Mohamadi A. Sarkar[1]

Nasal delivery is a potential alternative for systemic availability of drugs restricted to intravenous administration, such as peptide and protein drugs. Although nasal delivery avoids the *hepatic first-pass effect,* the enzymatic barrier of the nasal mucosa creates a *pseudo-first-pass effect.* The xenobiotic metabolic activity in the nasal epithelium has been investigated in several species including humans. The Phase I, cytochrome P-450 enzymes have been studied extensively for their toxicological significance, since these enzymes metabolize inhaled pollutants into reactive metabolites which may induce nasal tumors. The cytochrome P-450 activity in the olfactory region of the nasal epithelium is higher even than in the liver, mainly because of a three- to fourfold higher NADPH–cytochrome P-450 reductase content. Phase II activity has also been found in the nasal epithelium. The delivery of peptides and proteins has been hindered by the peptidase and protease activity in the nasal mucosa. The predominant enzyme appears to be aminopeptidase among other exopeptidases and endopeptidases. The absorption of peptide drugs can be improved by using aminoboronic acid derivatives, amastatin, and other enzyme inhibitors as *absorption enhancers.* It is possible that some of the surfactants, e.g., bile salts, increase absorption by inhibiting the proteolytic enzymes. Thus, in addition to the permeation barriers, there also exists an enzymatic barrier to nasal drug delivery, which is created by metabolic enzymes in the nasal epithelium.

KEY WORDS: nasal metabolism; nasal cytochrome P-450; enzyme inhibitors; absorption enhancers.

INTRODUCTION

Until recently antibiotics, antiinflammatory steroids, and decongestants were administered intranasally only for their local action, e.g., nasal decongestion and bronchodilation. The observation that systemic side effects appeared in some cases led to the conclusion that the nasal mucosa permits the systemic availability of some drugs. Nasal delivery offers a promising alternative to parenteral administration of drugs that cannot tolerate the rigorous gastrointestinal environment after oral administration. Nasal administration is being actively investigated as one of the possible noninvasive alternatives to delivering peptide and protein drugs.

The nasal epithelium has a defensive enzymatic barrier against the entry of xenobiotics, and the original concept of nasal delivery without first-pass effect is no longer applicable. Although the blood circulation from the nose is not presented to the liver, the nasal mucosa itself is a barrier to the direct systemic access of some drugs. Drug metabolism in the nasal mucosa is an important consideration not only in nasal drug delivery, but also for toxicologic implications because of xenobiotic metabolism of inhaled environmental pollutants or other volatile chemicals. The Phase I cytochrome P-450 enzymes can convert some of the airborne chemicals to reactive metabolites which may be involved in forming DNA adducts, increasing the risk of carcinogenesis in the nasopharynx and lung. Before the metabolic aspects of the nasal mucosa are considered, a brief review of the nasal anatomy and physiology is necessary.

Anatomy and Physiology of the Nose

The primary function of the nose is olfaction, but it also filters airborne particulate and heats and humidifies inspired air. The external portion of the nose does not have any significance in drug metabolism. The lateral wall of the internal nose contains the turbinates, the draining orifices of the paranasal sinuses, and the nasolacrimal duct. The nasal passageways are extremely convoluted, the turbinates divide the air spaces into thin slits only a few millimeters wide, and the surface area of the nasal mucosa is considerably increased (1). In adult humans, the nasal cavities are covered by a 2- to 4-mm-thick mucosa (2), the nasal cavity's volume is about 20 ml, and its total surface area is about 180 cm^2 (1).

Ethmoturbinates. This term is often seen in the nasal metabolism literature. The ethmoid bone is the principal supporting structure of the nasal cavities. The ethmoturbinates are composed principally of the olfactory mucosa. The olfactory receptors (Fig. 1) lie in the superior nasal turbinates (ethmoturbinates), also called the olfactory region. The olfactory epithelium of several species is abundant in cytochrome P-450 enzymes and is capable of metabolizing drugs (3). Below the olfactory region, the membrane contains capillaries and pseudostratified ciliated columnar cells with many goblet cells and ducts of Bowman's glands.

Maxilloturbinates. This is another term frequently encountered in literature. The paired maxillae unite to form the

[1] School of Pharmacy, West Virginia University, Health Sciences North, Morgantown, West Virginia 26506.

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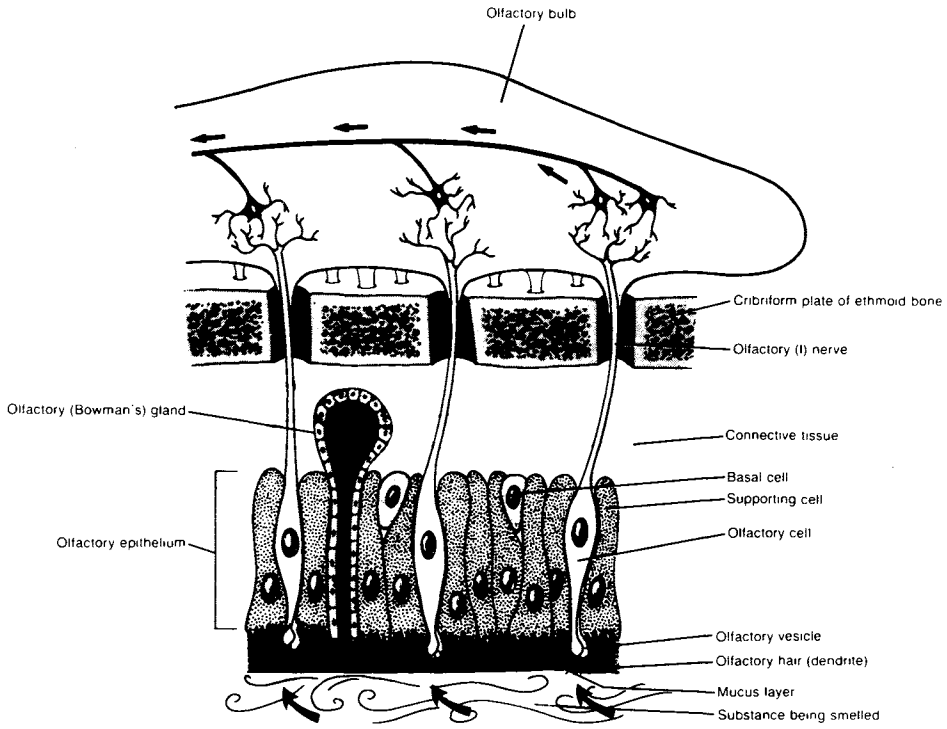

Fig. 1. Schematic representation of the olfactory receptors. (Reproduced from G. J. Tortora, *Principles of Human Anatomy,* Harper & Row, New York, p. 559.)

part of the lateral wall and floor of the nasal cavity. The maxilloturbinates form the respiratory mucosa; the pseudostratified ciliated columnar epithelium (Fig. 2) is often called respiratory epithelium. The yellow-brown olfactory epithelium usually has substantially higher metabolic capabilities than does the pinkish-white respiratory epithelium. Mucus-secreting goblet cells are interspersed within the nasal mucosa with ciliated cells, while the submucosa is rich in both serous and mucus glands. There is a continuous flow of mucus within the portions of the nose lined by ciliated respiratory epithelium, as a result of the ciliary activity, most of which is directed posteriorly toward the nasopharynx. The area below the epithelium is a highly vascularized connective tissue region.

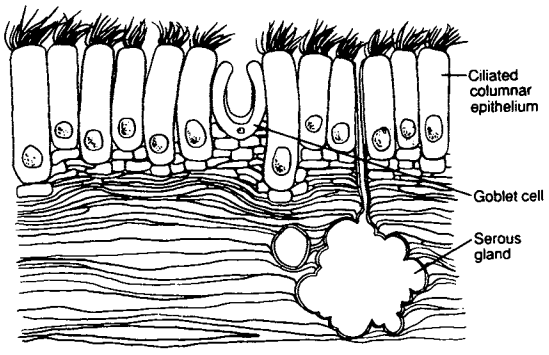

Fig. 2. Schematic representation of the respiratory mucosa consisting of the pseudostratified columnar epithelium. This histological section shows the typical appearance of the nasal mucosa. The surface cells are largely ciliated columnar cells, interspersed with goblet cells. Mucus and serous secreting glands are found in the underlying loose and vascular submucosa. (Reproduced from K. G. Marshall and E. L. Attia, *Disorders of the Nose and Paranasal Sinuses,* PSG, Littleton, Mass., p. 9.)

Absorption of drugs across the nasal mucosa results in direct systemic exposure, thus avoiding the first-pass hepatic metabolism associated with oral administration. *However, an alternative first-pass effect is created, by the metabolic activity within the nasal mucosa.* As the existence of drug metabolizing enzymes in the nasal mucosa of humans has not been definitively established, the information provided in this article relates to different animal species. Metabolism in the nasal mucosa has been investigated in rat, rabbit, Syrian hamster, dog, and monkey (3). It would be reasonable to assume that despite some interspecies variation seen in the nasal drug metabolizing enzymes, humans may also have a similar profile of enzymes in the nasal mucosa. Such a comparison can be justified by a report on metabolism by human nasal microsomes (4), where the results were comparable to the observations in animals (5). This speculation is supported by the vast information available on drug metabolizing enzymes in the liver and the fact that, despite some interspecies differences, most of the hepatic enzymes studied in animals are homologous in humans.

The various drug metabolizing enzymes identified in the nasal mucosa are oxidative Phase I enzymes, conjugative Phase II enzymes, and proteolytic enzymes.

OXIDATIVE PHASE I ENZYMES

Cytochrome P-450

The nasal epithelium is recognized as a potential first line of defense of the lung against airborne xenobiotics. It is

constantly exposed to the external environment and is one of the major target organs for toxicity of many inhaled substances. For example, many environmental pollutants adsorbed onto particles (of sizes 1- to 100-μm mass median aerodynamic diameter) have been observed to deposit in the nasopharyngeal region of the respiratory tract (6).

Studies of biotransformation of foreign compounds in the nasal mucosa of animals have shown extensive P-450-dependent metabolism. Some cytochrome P-450 enzymes have been linked to increased risk of cancer and/or other toxicological effects such as lesions in the nasal mucosa, because of formation of reactive metabolites which can form mutagenic DNA adducts. Nasal cancer has been reported to occur in experimental animals after inhalation exposure to a variety of important industrial chemicals, including formaldehyde (at an exposure of 15 ppm) (7), hexamethylphosphoramide (8), and the tobacco-specific nitrosamines (9). The common air pollutant, benzo(*a*)pyrene (BaP), has been shown to be converted to the carcinogenic metabolite BaP-7,8-diol-9,10-epoxide in the rat nasal tissue (10). Inhalation studies with benzo(*a*)pyrene in Syrian hamsters resulted in neoplasms in the nasal cavity, larynx, pharynx, esophagus, and forestomach (11). The gastrointestinal tract tumors could possibly be a result of swallowing of the mucus laden with BaP or its reactive metabolites. Nasal administration is not necessary to initiate the carcinogenic effect of P-450 metabolism. Long-term experiments have indicated that oral administration of large doses of phenacetin to rats will induce carcinomas of the nose (12).

Numerous other compounds have been shown to be metabolized *in vitro* by the nasal P-450-dependent monooxygenase system, e.g., nasal decongestants, essences, anesthetics, alcohols, nicotine, and cocaine (13). A number of these compounds are also metabolized *in vivo* in the nasal mucosa, as demonstrated by whole-body autoradiography (14). The results from these studies indicate that the nasal mucosa is, in many cases, much more active (on a per milligram of protein basis) than other organs, including liver, in the metabolism of foreign compounds both *in vitro* and *in vivo*.

The specific content of the P-450 in nasal mucosa is relatively high, second only to that of the liver. *The most striking feature is that the catalytic activity of the P-450 enzymes in the nasal epithelium is higher than in any other tissue including the liver.* The relatively high levels of the cytochrome P-450 enzymes and the maximal catalytic activity serve as a protective mechanism against the constant barrage of xenobiotics. The higher activity could be a result of a higher NADPH–cytochrome P-450 reductase content, which is the rate-limiting factor in the cytochrome P-450 oxidoreductase cycle, since the reductase is involved in the transfer of the first or the second electron in the cycle (15). Immunoblot analysis with antireductase IgG has shown that rabbit nasal microsomes have very high levels of cytochrome P-450 reductase (16). The ratio of NADPH–cytochrome P-450 reductase to cytochrome P-450 content is 1:11 to 1:15 in the liver, whereas in the olfactory region of the nasal epithelium this ratio is 1:2 to 1:3 (17). The higher proportion of NADPH reductase makes the cytochrome P-450 enzymes more efficient, thereby considerably increasing the catalytic activity.

The presence of cytochrome P-450-dependent monooxygenases has been reported in the nasal tissue of rabbit, guinea pig, rat, Syrian hamster, mice, and dog (Table I). The highest microsomal cytochrome P-450 concentration was found in the Syrian hamster nasal epithelium, and in general, for all the species the highest microsomal cytochrome P-450 concentration was found in the ethmoturbinates (olfactory region). Subsequently the catalytic activity (e.g., aminopyrine N-demethylation) is more pronounced in the mucosa of the olfactory region than in the mucosa covering the respiratory region (18). In another study (17) catalytic activity, using four substrates, was compared between the liver and the nasal olfactory epithelium (Table II). All four substrates were metabolized more rapidly in the olfactory epithelium, probably because of higher NADPH cytochrome P-450 reductase activity, which was two- to threefold higher in the olfactory epithelium than the liver. This observation was consistent across various species and sex (17).

All three major classes of hepatic inducible cytochrome P-450 isozymes have been found in the nasal mucosa. The phenobarbital (PB)-inducible P-450 isozymes are concentrated within the respiratory epithelium, whereas the 3-methylcholanthrene (MC)-inducible P-450 isozymes are located in the Bowman's gland of the olfactory region. The pregnenolone-16α-carbonitrile (PCN)-inducible P-450 isozymes are distributed uniformly throughout the nasal mucosa (19). Two unique cytochrome P-450 isozymes, NMa and NMb, related to P-450 3a, have also been purified and characterized in the rabbit nasal microsomes (20). Oxidative metabolism is thought to play an important role in olfaction.

Drug Metabolism in Human Nasal Mucosa

Few reports are available on human nasal metabolism capabilities (4,5). However, these observations strongly suggest that significant metabolic activity is present in the human nasal mucosa. The volatile diethylnitrosamine (DEN) present in air, water, foods, and tobacco smoke is metabolized upon inhalation and, thus, presents a high risk of nasal tumors to individuals working in the leather or wood industry (21). Investigation of catalytic activity (4) showed that the DEN-deethylase activity of human nasal mucosa microsomes was 1.06 ± 0.99 nmol/mg/min (n = 4) and that of human liver microsomes was 2.7 ± 1.44 nmol/mg/min (n = 5). Since the P-450 content in the human liver is 0.2–0.8 nmol/mg protein (22) and 0.026 nmol/mg protein in the hu-

Table I. Cytochrome P-450 in Nasal Mucosa[a]

Animal	Conc. cytochrome P-450 (pmol P-450/mg microsomal protein) Total nasal tissue[b]
Syrian hamster	460 ± 8 (12)
Cynomolgus monkey	420
Rabbit	350 ± 71 (3)
Dog	235 ± 43 (3)
Rat	110 ± 15 (9)
Guinea pig	94 ± 6 (12)
Mouse	65 ± 6 (30)

[a] Data presented from Ref. 3.
[b] Number of animals in parentheses; results are mean ± SE.

Table II. Drug Metabolism in Microsomes Isolated from Liver and Olfactory Epithelium of Male Hamsters[a]

Metabolic activity (nmol/mg protein/min)	Liver	Olfactory epithelium
Cytochrome P-450 (nmol/mg protein)	1.15 ± 0.19	0.58 ± 0.14
7-Ethyoxycoumarin deethylase	3.74 ± 0.58	27.10 ± 11.9
7-Ethoxyresorufin deethylase	0.21 ± 0.05	0.56 ± 0.10
Hexobarbitone oxidase	1.4	45.9
Aniline hydroxylase	0.95 ± 0.15	4.67 ± 0.73

[a] Data reproduced from Ref. 17. Results are mean ± SD of at least three observations, each from tissues pooled from at least two animals. Hexobarbitone oxidase results are means of two observations.

man respiratory nasal mucosa (5), the DEN-deethylation activity of nasal mucosa, expressed per nanomole of P-450, is 2–10 times higher than that of human liver.

The nasal mucosa, in particular the olfactory region of the nasal cavity, is rich in the cytochrome P-450 enzymes that metabolize xenobiotics. This "first-pass effect" of the nasal mucosa should be taken into considerations for drugs to be delivered by the nasal route. The high oxidative metabolic capabilities of the nasal mucosa should also be taken into account in toxicokinetic models intended to predict the fate of inhaled compounds.

Steroid Metabolism by Nasal Mucosa

Alternative routes of administration of steroids are needed especially for progesterone and testosterone, because of their poor oral absorption. The nasal bioavailability after *in vivo* administration of the same dose of progesterone to rats was 100% (relative to iv), compared to only 1.2% for intraduodenal bioavailability (23). However, conflicting results have been observed in *in vitro* studies in the rat nasal mucosa. Extensive metabolism and uptake of progesterone and testosterone are noted in the rat nasal mucosa *in vitro* (24). The pattern of metabolites shows that these steroids are reduced as well as hydroxylated at multiple positions. This discrepancy between the *in vitro* and the *in vivo* results could be due to rapid uptake of progesterone by the respiratory mucosa, which is less abundant in metabolizing enzymes than the olfactory mucosa.

In addition to the cytochrome P-450 enzymes, the following oxidative Phase I enzyme activity have also been studied in the nasal epithelium.

Flavin-Containing Monooxygenases and Aldehyde Dehydrogenases

The monooxygenase activity is high in the nasal ethmoturbinates of rabbit respiratory tract in the area of the olfactory mucosa, with levels exceeding those found in the liver (25). Aldehyde dehydrogenases I and II and formaldehyde dehydrogenase have also been detected in the nasal cavity of the rat (26). The specific activities of aldehyde dehydrogenase II and formaldehyde dehydrogenase in homogenates of olfactory epithelia were higher than in respiratory epithelial homogenates.

Epoxide Hydroxylase

The reactive epoxides generated by the cytochrome P-450 enzymes are inactivated by hydrolysis, hence it is not surprising to find epoxide hydroxylase in olfactory and respiratory epithelia, Bowman's glands, and seromucous glands, the respiratory region having the highest levels of epoxide hydroxylase. The epoxide hydroxylase activity of rat nasal tissue tends to reflect that reported for rat liver (10,27).

Carboxylesterase

These enzymes can be of special importance for delivery of drugs with carboxylic acid esters as functional groups. The interest in this enzyme is generated because of the development of lesions in the nasal mucosa after exposure to certain volatile industrial esters, e.g., solvents used in paint and coating industries. The ability of rodent nasal mucosa to hydrolyze these and a series of other esters to acid and alcohol metabolites has been studied (28). For certain substrates, the rate of hydrolysis is equivalent to or greater than that found when using tissue preparations from liver (29). The activity of olfactory mucosal carboxylesterases has been shown to be three to six times greater than respiratory mucosa when *p*-nitrophenyl butyrate is used as a substrate (29).

Carbonic Anhydrase

Carbonic anhydrase, the zinc-containing enzyme that accelerates the rate of formation of carbon dioxide from carbonic acid, is present at a high concentration in rat olfactory cells but absent in supporting cells. Besides its normal function with carbonic acid as a substrate, carbonic anhydrase exhibits considerable simple esterase activity and may be responsible for a major part of the olfactory esterase activity (30).

CONJUGATIVE PHASE II ENZYMES

Comparative evaluation of nasal metabolism of 17β-estradiol indicated that significantly more conjugation occurred when the drug was administered via the nasal route compared with the iv route (31). The extent of conjugation following nasal administration of 17β-estradiol decreased as the dose increased, suggesting that the drug is conjugated within the nasal mucosa and that this process is saturable. The authors did not determine the specific conjugated metabolites, hence it is not possible to evaluate which of the conjugation pathways is saturated.

Glucuronyl and Sulfate Transferase

Studies utilizing pooled nasal tissues from the maxilloturbinates, ethmoturbinates, and nasal epithelial membrane has shown the presence of these enzymes (32).

Glutathione Transferase (GST)

This is a Phase II detoxifying enzyme converting the electrophilic reactive metabolites, formed by Phase I enzymes, into harmless glutathione conjugates. Thus, GST is

important for the inactivation of inhaled mutagens and carcinogens. Reports indicate that a significant level of GST activity is located in the cytosol of olfactory and respiratory mucosa in humans. The specific activity was higher than that reported for a number of extrahepatic tissues (33), suggesting the potential of nasal mucosa in protection of the body against the toxic effect of compounds present in the inhaled air. The activities of the Phase II enzymes may be dependent upon the activated cofactor in these tissues (10,27). Quantities of glutathione, on a per gram tissue basis, in rat nasal tissue were found to be about one-tenth those typically reported in rat liver. Thus, the reactive intermediates formed by the cytochrome P-450 enzymes could accumulate when available glutathione is exhausted by conjugation reactions.

The above Phase I and Phase II enzymes have been studied in the nasal mucosa only for their toxicological importance, since it is the site for producing toxic metabolites from the inhalation exposure of environmental pollutants. This hypothesis seems very likely, since the cytochrome P-450 activity is higher in the nasal mucosa than any other site in the body, even the liver.

This information can be useful when formulating a drug for nasal delivery. The earlier theory, that the nasal delivery eliminates first-pass metabolism in the liver, can be refuted, since the nasal mucosa could itself present a first-pass effect. The presence of esterases (28) in the nasal mucosa provides a unique opportunity to avoid the nasal first-pass effect by designing ester prodrugs for nasal delivery. The product would be rapidly absorbed through the nasal mucosa and metabolized by the esterases, and the parent compound would be available in the systemic circulation.

PROTEOLYTIC ENZYMES AND THEIR IMPACT ON DELIVERY OF PEPTIDE AND PROTEIN DRUGS IN THE NASAL MUCOSA

The oral delivery of peptides and proteins has not been successful, primarily because of extensive digestion of these substances by protease and proteinases in the gastrointestinal tract. Consequently, alternative routes, e.g., via the nasal mucosa, which are presumed to be deficient in these enzymes, are being investigated for peptide and protein delivery. Some peptides can be absorbed in a systemically effective quantity following transnasal administration (see Ref. 34 and references therein). On the other hand, variable and low systemic absorption has also been reported for polypeptide hormones with a large molecular size, such as insulin (34) and leutinizing hormone releasing hormone (35). Although the bioavailability of peptides and proteins from the nasal mucosa is substantially improved over the oral route, it is still far from optimal when compared to intravenous routes. This result may be attributed to the resistance encountered by peptides and proteins in penetrating the nasal mucosa, as well as the susceptibility to degradation by proteases and proteinases that may be present in the mucosa. The inadequate bioavailability of peptides and proteins even in the presence of penetration enhancers suggests that there is another barrier, an enzymatic barrier, which limits absorption. The mucosal membranes of the nasal cavity are known to have various types of peptidase and protease activities, including both exopeptidases and endopeptidases (36). Lee and co-workers (37,38) demonstrated that the proteolytic activities in homogenates of the mucosal tissues of the albino rabbit against methionine enkephalin, [D-Ala^2]methionine enkephalinamide, substance P, insulin, and proinsulin were comparable to those in the ileum. On evaluation of the metabolism of the peptides enkephalin, leucine-enkephalin, and met-enkephalinamide in homogenates of nasal mucosa, it was found that all the peptides disappeared rapidly (39), presumably because of the action of aminopeptidases, dipeptidyl peptidase, and dipeptidyl carboxypeptidase. Based on the fractions of amino acids obtained after metabolism of enkephalins, the authors felt that aminopeptidases were the principal peptidases mediating the hydrolysis of enkephalins. Thus, inhibition of the aminopeptidase should minimize enkephalin degradation. The authors cautioned, however, that the structural organization of the peptidases that contribute to the hydrolysis of enkephalins and other peptides and proteins can be destroyed upon homogenization. It is conceivable that significant differences in the susceptibility of a given peptide to hydrolysis could be discerned *in vivo* if the peptidases have different structural organization (39).

In conclusion, aminopeptidase activity in the nasal mucosa has been found to be similar to that of the ileal mucosa in its subcellular distribution. Specifically, almost half of the aminopeptidase activity in the nasal mucosa of the albino rabbit is membrane bound (40). It has yet to be determined whether the *membrane-bound* aminopeptidases are active enough to degrade completely most of the peptides and proteins and whether the *cytosolic* aminopeptidases are active enough to degrade the peptides and proteins that have escaped hydrolysis in the membrane. A complete understanding of the enzymatic barrier is lacking, thus impeding effective delivery of peptides and proteins through the nasal mucosa.

PROTEOLYTIC ENZYME INHIBITORS AS ABSORPTION ENHANCERS

Since the major enzyme causing a barrier to absorption of peptides from the nasal mucosa are aminopeptidases, inhibition of peptidases should improve the absorption of peptide and protein drugs susceptible to this group of enzymes. This hypothesis has been evaluated with considerable success. Various aminoboronic acid derivatives have been shown to be potent peptidase (specifically aminopeptidase) inhibitors (41,42). These compounds have potential utility for improving the delivery of peptide drugs which are metabolized by aminopeptidases. Thymopentin, a pentapeptide with immunomodulatory activity, rapidly disappeared (elimination half-life, 12 min) after *in vivo* nasal perfusion, and two metabolites, Lys.Asp.Val.Tyr and Asp.Val.Tyr, appeared in the perfusate. However, in the presence of 1 μM boroleucin, an aminopeptidase inhibitor, the disappearance half-life was prolonged by more than threefold, to 37 min. The concentration of Lys.Asp.Val.Tyr, the product of aminopeptidase metabolism, was also reduced. These results demonstrate that thymopentin is metabolized by nasal mucosal aminopeptidases and that its metabolism can be inhibited by boroleucine (43). In other studies using leucine enkephalin as a model substrate, it has been shown that these aminoboronic acids inhibit metabolism by the nasal mucosal

membrane (44). *In vitro* results indicate that aminopeptidases can be controlled by bestatin and puromycin (38) and that endopeptidase 24.11 and cysteine proteinase can be controlled by 1,10-phenanthroline and *p*-hydroxymercuribenzoate, respectively (45). Further nasal absorption of human growth hormone in the rats was substantially increased by amastatin (Fig. 3) (46), which inhibits aminopeptidase A among other specific aminopeptidases (47). However, bestatin, which inhibits aminopeptidase B, did not improve the bioavailability of the growth hormone. Such reports will help in identifying which specific isoforms of the peptidases metabolize specific substrates in the nasal mucosa of mammals. Thus, the selective inhibition indicates that aminopeptidase B may not be involved in the metabolism of the growth hormone. Addition of the proteolytic enzyme inhibitor aprotinin (trypsin inhibitor) or bacitracin (aminopeptidase inhibitor), coadministered with sodium taurodihydrofusidate, (STDHF), to the nasal formulations did not lead to any further increase in insulin absorption (48). However, it is possible that the metabolic degradation of insulin is negligible in nasal formulations already containing STDHF. Treatment of isolated perfused rat lung with a combination of the enzyme inhibitors, captopril and bestatin, augments the pressor effect of the pentapeptide, Leu-enkephalin (49); this combination has not been explored in nasal delivery of Leu-enkephalin, which is significantly metabolized upon nasal perfusion (50). Interestingly, this enzymatic degradation was considerably reduced by the addition of a large excess of di- or tripeptide containing either a tyrosine or a phenylalanine unit, suggesting that coadministration of a pharmacologically inactive peptide which competes as a substrate for the nasal peptidases may be a useful approach to improving the bioavailability of nasally administered peptides.

In the normal state, there appears to be a dynamic equilibrium among naturally occurring proteinases, peroxidases, and proteinase inhibitors, which may be disturbed by an excess of proteinases. The possible long-term consequences of disturbing this balance between enzymes and inhibitors in nasal drug delivery systems are not known.

ARE SOME PERMEATION ENHANCERS PROTEOLYTIC ENZYME INHIBITORS?

The nasal route is a potential method of administration for proteins and peptides. Since polypeptides are poorly absorbed from the nasal cavity, absorption enhancers are employed in attempts to increase the extent of peptide absorption. Examples of these enhancers are bile salts (51), chelating agents (45), surfactants (52), and fatty acids (53). Although the precise mechanism of action of the absorption enhancers is not known, it is speculated that these agents promote drug absorption by either (a) increasing membrane fluidity (54), (b) expanding the dimension of the paracellular pathway to solute transport (55), or (c) reverse micelle formation in the cell membrane, creating transient pores (57). An additional contributing mechanism proposed to explain the permeation enhancement properties of certain surfactants is their activity as protease inhibitors. Both Hirai *et al.* and Stratford and Lee demonstrated the inhibitory effects of certain bile salts on aminopeptidases (38,52). Stratford and Lee examined the *in vitro* protease activity of the nasal mucosa of rabbits by isolating and separating the cellular components of the membrane. In general, these studies show that the nasal mucosa does have protease activity that can be inhibited by the bile salts or sodium taurodihydrofusidate but that it may be a significant factor only at low peptide or protein concentrations. In the cases of insulin and human growth hormone, where high doses of protein are required, the protease inhibitory effects of certain surfactants cannot explain the increased absorption across the nasal mucosa. The same surfactant, sodium glycocholate, provides a bioavailability of 100% for corticotrophin-releasing hormone (CRH) but only 7.1% for growth hormone releasing hormone (GHRH). Such an observation cannot be explained solely based on increased membrane permeability; it is probable that additional factors are involved, such as a different susceptibility of different peptides to lytic enzymes which could be selectively inhibited (56). It has also been suggested that sodium glycocholate exhibits its penetration enhancing action by inhibiting leucine aminopeptidase activity, thereby protecting insulin from proteolysis in rat nasal homogenates. Compared to the most potent aminopeptidase inhibitors—bestatin and amastatin—sodium glycocholate is not as potent (58). However, it has a potency similar that of the other aminopeptidase inhibitors—puromycin and *p*-chloromercuribenzoate. It has also been suggested that bile salts appear to inhibit proteolytic activity by denaturing the enzyme and preventing the enzyme–substrate complex formed to undergo the necessary conformational change that aligns the catalytic site on the protease with the susceptible bond of the substrate (58). In addition, reports on administration to humans of calcitonin and glucagon with different absorption enhancers (59) indicated that one of the mechanism for enhancing the absorption of the peptides is inhibition of proteases. The authors indicated that, relative to glucagon, calcitonin required a very low concentration of the surfactants, hence the mechanism of absorption enhancement was probably enzyme inhibition. Entrapment of the peptide or protein in micelles of the surfactant-type penetration enhancers, thereby protecting it from the proteolytic enzymes, is another possibility that needs to be explored (45).

It is possible that the protease inhibitory effect acts in synergism with the increase in membrane permeability effects, which for some proteins could be the dominating mechanism, since either the enzymes inhibited by the sur-

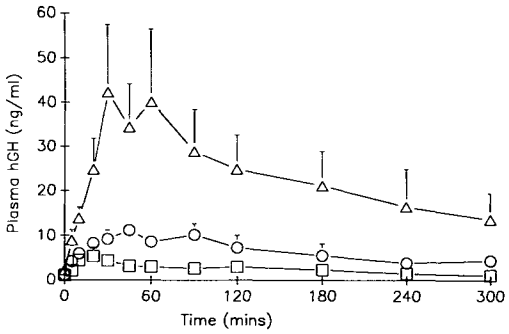

Fig. 3. Peptide inhibitors as absorption enhancers. Mean plasma levels of hGH (ng/ml) (n = 4) following intranasal administration of 1 mg/kg hGH solution alone (○) and in combination with two aminopeptidase inhibitors, 0.015% amastatin (△) and 0.015% bestatin (□). (Reproduced from Ref. 46.)

factants are not involved in the metabolism of the protein or the proteolytic enzymes are not present in the nasal mucosa.

The present strategy to facilitate mucosal peptide and protein absorption has been the coadministration of a peptide or protein with a penetration enhancer. Since all penetration enhancers promote peptide and protein absorption by perturbing membrane integrity, it is inevitable that varying extents of insult would occur to the mucosal tissues that are in contact with the enhancer (45). Many absorption enhancers, particularly the bile salts and nonionic surfactants, alter the membrane integrity and can permanently damage the nasal membrane (52). Consequently, these materials are unacceptable for chronic use in humans. These problems can be solved by selecting a suitable enzyme inhibitor as an absorption enhancer to deliver the peptides through the nasal mucosa without harming the mucosa. Therefore considerable attention is directed toward the use of enzyme inhibitors to facilitate absorption across the nasal mucosa. Although the long-term effects have not been evaluated, these "enzyme inhibitor/absorption enhancers" do not cause the side effects of nasal irritation, lesions, or damage of the membrane often seen with conventional surfactant absorption enhancers. An appreciation of the enzymatic barrier posed by the nasal mucosa can help in improved nasal delivery of proteins and peptides.

Nonmetabolic Clearance in the Nasal Mucosa

In addition to the metabolic clearance, rapid mucociliary clearance of the administered dose from the site of administration is another barrier limiting the absorption of peptide and protein drugs. Nasally administered drugs are usually cleared from the site of deposition within 30 min (60).

CONCLUSIONS

Metabolic clearance of substances from the nose into the blood is highly variable, depending on the particular compound considered. Thus, progesterone (23) and propranolol (63) tend to clear from the nose into the blood without undergoing metabolism, but nafarelin acetate seemed to clear poorly, perhaps as a result of metabolism (61). In the absence of a systematic study of the disposition of nasally instilled materials, the fate of inhaled xenobiotics cannot be accurately predicted. It is rather interesting that propranolol, which suffers an extensive gastrointestinal and hepatic first-pass metabolism after oral administration (62), is rapidly absorbed unmetabolized after intranasal administration (63). The AUCs after equivalent intravenous and nasal doses of propranolol are identical, indicating the efficiency and rapidity of absorption from the nasal mucosa. The bioavailability of 10 mg of propranolol hydrochloride was identical after intravenous and nasal application (compared to 25% for oral dose). The reason for the lack of extensive "first-pass" effect in the nasal mucosa for drugs such as propranolol is that only a few specific cytochrome P-450 isozymes are present in the nasal epithelium, necessary to maintain a biochemical defense to protect the lungs from harmful reactive metabolites. Other isozymes of cytochrome P-450 and Phase II conjugative enzymes involved in the metabolism of xenobiotic drugs are probably nonexistent or not developed.

Whereas intranasal therapy cannot completely replace iv therapy, it can be used along with other routes or as alternative therapy in chronic iv therapy. Salzman *et al.* (64) have reported the successful use of aqueous nasal applications of insulin in patients over a period of 6 months as an adjunct to subcutaneous therapy. This approach to drug delivery is now being extended to other polypeptides (e.g., LH-RH, interferons, and influenza vaccines). Therefore nasal delivery of peptides, proteins, and other drugs is a viable option, but before extensive research is done to explore this route for delivery potential, an initial screening, in nasal homogenates, of possible metabolism would provide valuable information regarding the usefulness of delivering the drug by the nasal route.

In vitro methods can be used to evaluate the metabolism of the compound that is considered for nasal delivery before spending time, effort, and resources on conducting expensive *in vivo* experiments. Nasal homogenates have been used successfully to study metabolism of drugs in the nasal mucosa (33). Tissue culture techniques can also be used to study the metabolism and transport of peptides and proteins across epithelial cells.

Recent studies have indicated that surfactants such as sodium glycocholate may facilitate increased absorption by damaging the nasal membrane and producing large fissures through which the peptide molecule are able to penetrate. The problems of local irritancy and subsequent damage can be overcome by avoiding surfactants as absorption enhancers and replacing them with proteolytic enzyme inhibitors to increase the absorption of peptide drugs. The knowledge of the metabolizing enzymes, especially the esterases and proteolytic enzymes, can be used to design a novel delivery system. A combination of a peptide prodrug (such as an ester) and a peptidase inhibitor may provide a useful approach to the enhancement of peptide absorption from the nasal cavity.

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