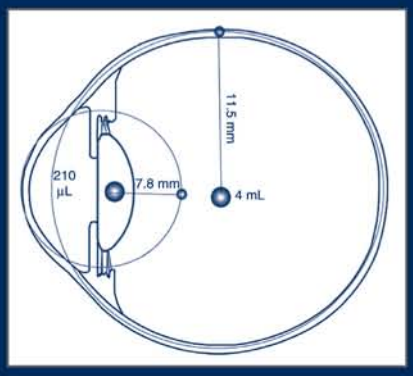
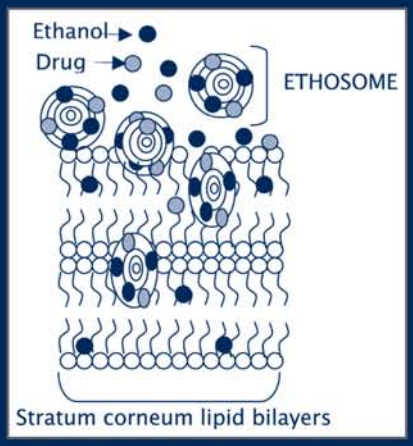
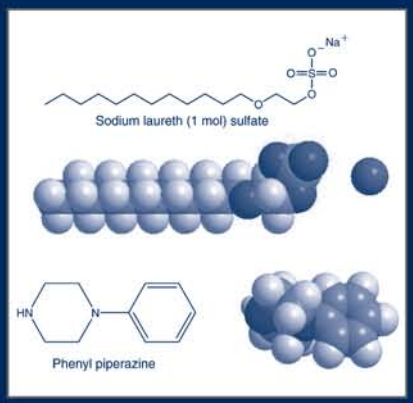
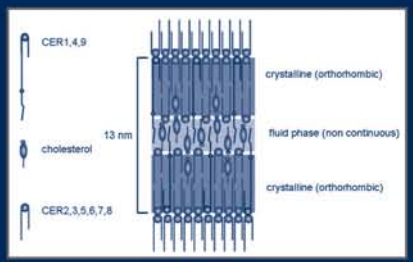
Enhancement *in* Drug Delivery









Edited by
Elka Touitou
Brian W. Barry

CRC Press
Taylor & Francis Group

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an informa business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

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No claim to original U.S. Government works
Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 0-8493-3203-6 (Hardcover)
International Standard Book Number-13: 978-0-8493-3203-6 (Hardcover)

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Library of Congress Cataloging-in-Publication Data

Enhancement in drug delivery / edited by Elka Touitou, Brian W. Barry.
p. ; cm.
Includes bibliographical references and index.
ISBN 0-8493-3203-6
1. Drug delivery systems. 2. Drugs--Dosage forms. 3. Drugs--Physiological transport. 4. Absorption (Physiology) I. Touitou, Elka, 1942- II. Barry, Brian W., 1939-
[DNLM: 1. Drug Administration Routes. 2. Adjuvants, Pharmaceutic. 3. Drug Delivery Systems. WB 340 E58 2006]

RS199.5.E54 2006
615'.1--dc22 2006045582

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Preface

Enhancement in drug delivery and absorption via a specific route of administration often becomes essential in the design of novel pharmaceutical products and new therapies. On the one hand, biotechnological therapeutic molecules usually require enhancers for their successful administration by noninjectable modes; alternatively, for the more efficient delivery of conventional drugs, other routes that overcome the disadvantages of traditional administration may also require additional augmentation.

The literature currently available on drug delivery enhancement, including the most recent developments, though extensive, is still fragmented into sources that concentrate on specific administration routes, making it difficult to gain an integrated knowledge in this field.

The concept that guided us while editing this book was that by assembling knowledge on various modes of enhancement for problematic administration routes, we could deliver a multidisciplinary comprehensive review to the readers. Thus, we believe that this volume can be used as a reference book for the research community and pharmaceutical industries as well as an educational tool for senior students and practitioners in the field of pharmaceutics, medicine, and health-related disciplines. More specifically, this book is targeted toward scientists in academia and industry and graduate students in various research-intensive programs in pharmaceutical sciences, biotechnology, and medicine who are dealing with many aspects of drug design, development, and testing.

Within the chapters, the reader may find the same enhancer tested for various administration routes and in diverse experimental models. By understanding the properties and behavior of the accelerants operating within such systems, the scientist may be inspired with new ideas for choosing the ideal promotor for a new application. The contributing authors, in their reviews, discuss not only the achievements, but also the failures and drawbacks of various enhancers that are often mainly related to their toxicities.

Thus, the scheme of this book is to present a comprehensive review of the theory and methods for enhancing drug absorption through various routes of the human body. It is hoped that, by a process of cross-fertilization, investigators primarily involved with one specific route of delivery will find additional stimulation and helpful concepts applicable to their own area of expertise from a reading of the approaches other workers have used within their spheres of activity. Not every enhancement method ever tried, however speculative, for each route of delivery finds a place in the relevant section. We believe that the book presents the most interesting approaches operating at the time the authors prepared their contributions.

The opening chapters deal with the major route of drug delivery, and reflect on gastrointestinal anatomy, physiology, and permeation pathways, together with such considerations as the role of surfactants in accelerating the input of macromolecules, targeted gastrointestinal delivery, inhibition of enzymes and secretory transport, problems with lipophilic drugs, and the use of chitosan and its derivatives. Treatments on permeation pathways in rectal absorption and relevant enhancers provide information for a route whose use varies significantly in different countries. A deliberation on the basic biopharmaceutics of buccal and sublingual absorption precedes a contemplation of the role of chemical enhancers. The problems associated with circumventing the barrier of the skin during transdermal drug delivery bring into sharp focus many problems associated with breaching biological barriers; the stratum corneum has developed an elegant structure with which to limit very significantly

the access of most chemicals, while permitting the controlled loss of water. It is therefore in this area of drug delivery that we find use of the widest range of enhancement methods. After a chapter orientating the reader to the structure and barrier function of the stratum corneum, contributors consider the most widely investigated enhancement approaches. These include the very extensive employment of chemical promotors, at least experimentally, electrically assisted methods such as iontophoresis, electroporation, and ultrasound, the promise offered by vesicular carriers, as well as methods that combine liposomes with electrical potentiation. The final transdermal chapter considers the approach of by-passing or removing the major source of our difficulties, the horny layer of the skin.

In recent years, a considerable interest in nasal delivery has developed and the text on the physiological parameters that affect this process leads once again to the use of chemical enhancers. The problem of peptide delivery, which is a concern with respect to all routes of delivery in light of the as yet unfulfilled promises of the biotechnology revolution, completes this section. Consideration of the nature of the vagina and uterus as absorbing organs sets the scene for a contemplation of strategies for improving the bioavailability of a drug when administered via the vaginal route. Details on the structure and function of the eye help us to understand delivery systems for this route and the relevance of chemical permeation enhancers, together with the promise of iontophoresis. The final section of the book deals with structure and function of the blood–brain barrier and strategies for overcoming it.

We express our appreciation to all the authors for contributing outstanding chapters. We would also like to thank Mrs. Yvonne Western and Mrs. Madelyn Segev for their excellent assistance with respect to the manifold duties involved in the preparation of the manuscript.

Elka Touitou
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Editors

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19 Physiological Factors Affecting Nasal Drug Delivery

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19.1 INTRODUCTION

The nasal route of drug delivery is used for the direct administration of medicines to the nose for treatment of local conditions or the systemic delivery of compounds that are not easily delivered by the oral route. It is also suggested that there may be a direct route for drug absorption to the central nervous system (CNS) from the olfactory region of the nose.

Nasal delivery has long been used to administer topically acting drugs to treat localized ailments such as nasal symptoms of common cold and allergy. Advantages of using the nasal route to treat local symptoms include the immediate targeting of relatively high drug concentrations, the simplicity of administration, reduced systemic exposure, and good patient acceptability. More recently, drugs with systemic actions have been marketed for nasal delivery in preference to oral delivery or injection. This takes advantage of the potentially rapid and high systemic availability of nasally administered compounds. Diseases or conditions for which nasal administration has been used to achieve such delivery include hormone replacement therapy (estradiol), osteoporosis (calcitonin), pain management (butorphanol, sumatriptan and zomitriptan), smoking cessation (nicotine), enuresis (desmopressin), endometriosis (nafarelin) and motion sickness (metoclopromide). The possibilities for CNS delivery via nasal administration are currently being investigated for the delivery of polar drugs to treat chronic CNS conditions such as Parkinson's disease or Alzheimer's disease.

19.2 ANATOMY AND PHYSIOLOGY OF THE NOSE

19.2.1 Nasal Passages

In humans, the nose is divided into two symmetrical left and right channels (passages) that are separated by the cartilaginous, bony, nasal septum. These parallel passages have distinct regions beginning with the vestibular area (nostrils), leading via the internal ostium (nasal valve) to the atrium (narrow transitional area), then to the main respiratory region (comprised of the nasal turbinates and olfactory region) and finally the nasopharynx where the channels converge (Figure 19.1) [1,2].

The lining of the vestibule, which contains a zone of short stiff hairs (vibrissae), acts as a baffle system for filtering out large (>10 μm) airborne particles. The stratified squamous epithelium of the vestibule is resistant to dehydration and inhaled irritants and poorly permeable. Between the vestibule and atrium, there is a constriction known as the internal

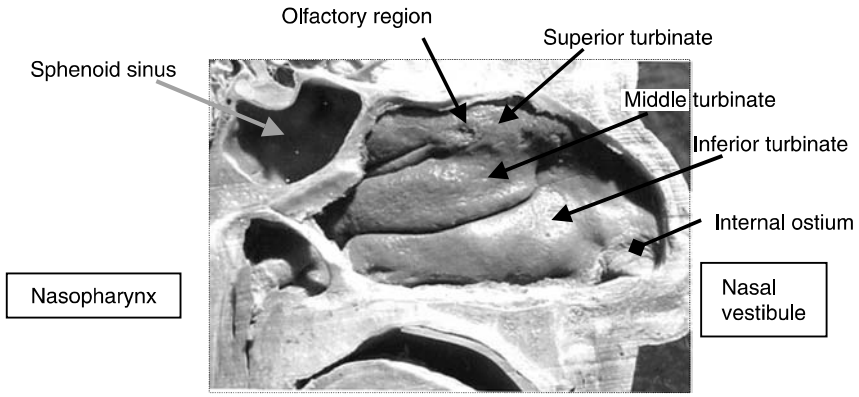


FIGURE 19.1 Lateral section of the nasal cavity, showing the extensive respiratory region (the turbinates) and the olfactory region. (From Jones, N., *Adv. Drug Deliv. Rev.*, 51, 5, 2001.)

ostium or nasal valve, which is the narrowest passageway of the entire airway (Figure 19.1). The incoming airstream is squeezed through this narrow passage, through the atrium, then curves into the main nasal passage—the respiratory region.

The respiratory region is formed of highly convoluted chambers (turbinates), which stretch from the atrium to the beginning of the nasopharynx. This region forms the middle and posterior thirds of the nasal cavity, is approximately 5–8 cm long, and is divided laterally into the superior, middle, and inferior turbinates. The olfactory region lies in this region and is situated on the roof of the nasal passage, accounting for 3%–5% of the surface area of the entire nasal cavity in humans. At a distance approximately 12–14 cm from the nostrils, the septum ends and the airways merge into the nasopharynx (Figure 19.1).

A primary function of the nasal cavity is olfaction. The location of the olfactory mucosa, recessed on the roof of the respiratory passage, means that it is accessed only by diffusion of volatile or airborne substances in inhaled air. The specialized epithelium features sensitive olfactory neurons that provide a direct link to the CNS. The structural features of the epithelium and potential for drug delivery are considered in detail in Section 19.6.1.

As normal breathing occurs via the nose, the nose also conditions air for inhalation for the lung. The anatomy of the nose permits intimate contact between inspired air and the mucosal surface, enabling air to be warmed and humidified by the rich blood flow and secretions of the epithelium. Air is also filtered in the nasal cavity, which is one of the most important physiological defensive mechanisms of the respiratory tract. It protects the body against any inhaled noxious materials or airborne contaminants such as dust, microorganisms, and allergens. The efficiency of particle removal is dependent upon a number of factors including the aerodynamic diameter of the inhaled particles. For example, as previously mentioned, particles greater than 10 μm are generally filtered out by the vibrissae at the nostrils, smaller particles (approximately 5–10 μm) are deposited in the nasal passages and subsequently cleared by the process of mucociliary clearance. Particles less that 2 μm are not normally filtered out and may enter the lungs.

19.2.2 Blood Supply

The nasal septum is supplied posteriorly by the sphenopalatine artery, superiorly by the anterior ethmoid artery, and antero-inferiorly by the superior labial branch of the facial artery. In addition, the inferior and lateral walls of the nasal cavity are supplied by the palatine and ethmoid artery, respectively, whereas the remainder of the blood supply to the nasal cavity comes from the sphenopalatine artery.

The direction of arterial blood flow in the nose runs anteriorly against inspiration. Blood vessels are arranged in such a manner as to provide an erectile capacity to the mucosa enabling the airway to widen and narrow. Blood flow through the autonomically controlled vasculature of the nasal tissue is of importance in the conditioning of inspired air.

19.2.3 Respiratory Epithelium

The nasal epithelium undergoes a transition in the atrium from the squamous epithelium of the vestibule to the more permeable epithelium of the respiratory region. The nonolfactory respiratory region is covered by typical airway epithelium, similar to that of the lungs. Basal, goblet, ciliated, and nonciliated cells form a pseudostratified, columnar, ciliated, highly vascular mucosa (Figure 19.2) [3].

The airway surface is formed by columnar ciliated and nonciliated cells interspersed with mucus-secreting goblet cells. The surface area of the epithelium is increased by the presence of numerous microvilli. The epithelial cells are held together at their apical surface by tight

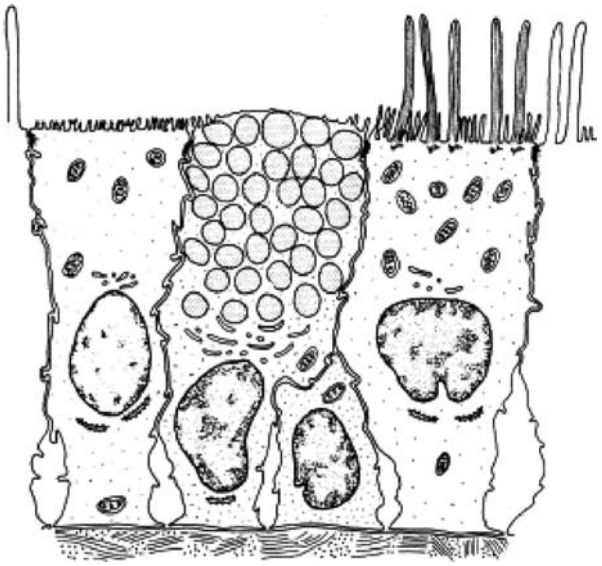


FIGURE 19.2 Diagrammatic representation of the cell types of the nasal mucosa as seen by transmission electron microscopy showing (I) nonciliated columnar cell, (II) goblet cell with mucus granules, (III) basal cell, and (IV) ciliated columnar cell. The epithelium is covered by a mucus layer 5–10 μm thick (not shown). (From Mygind, N. and Dahl, R., *Adv. Drug Deliv. Rev.*, 29, 3, 1998.)

junctions, which act as a physical barrier against the ingress of foreign material. The dimensions of these tight junctions provide a size-dependent barrier to the passage of larger compounds between adjacent cells (paracellular transport). Basal cells are located at the base of the epithelium and are precursors of columnar cells (ciliated and nonciliated) and goblet cells. The goblet cells are most dense in the posterior area of the nasal cavity and contain secretory granules in which dehydrated mucus glycoprotein is stored. The epithelium rests upon a layer of connective tissue fibrils called the basement membrane, underneath which is the submucosa (lamina propria), which contains a dense network of fenestrated capillaries that supply the tissue with a large volume of blood [4]. Glands lined by mucus and serous secretory cells are located in the submucosa with ducts leading to openings at the epithelial surface.

19.2.4 Mucus and Cilia

Nasal secretions consist mainly of heterogeneous secretory products originating from the goblet cells and submucosal glands, with additional contributions from lacrimal fluid and the vascular system. Together these secretions form a distinct two layers of airway lining fluid consisting of (i) a low-viscosity periciliary fluid layer bathing the epithelial cell surface and the cilia and (ii) a continuous mucous gel blanket overlying the periciliary fluid at the air interface. The periciliary fluid is a watery, ionic solution, maintained by transepithelial ion transport, which provides an environment within which the cilia are able to beat and perform their mucus clearing function [5]. The pH of periciliary fluid is 5.5–6.5 and any disruption to this can affect drug absorption, metabolism, toxicity, and physiological defense mechanisms.

The viscous mucus layer provides a physical protective barrier for the mucosa; trapping, binding, and clearing inspired particles. Approximately 1.5–2 L of mucus is secreted daily and the resultant gel layer varies between 5 and 10 μm in thickness. Mucus is composed of 95% water plus mucin glycoproteins (0.5%–5%), lipids in low proportions, mineral salts (1%), and

free proteins (0.5%–1%). The protein content of mucus includes immunoglobulins, lysozyme, lactoferrin, and other enzymes with the exact composition varying [6].

The secretory mucins are the major functional constituents of mucus [7]. Respiratory mucins are high molecular weight glycoproteins ($2–40 \times 10^6$ Da) that comprise long, linear, apparently flexible threads, which vary in length from 0.5 to 10 μm. They are composed of subunits (monomers) each of about 500 nm in length, joined end to end via disulfide bonds. A characteristic of mucin is the high carbohydrate content of the molecule (70%–90% by weight), occurring as neutral and acidic oligosaccharide units. These units are assembled from varying numbers of monosaccharides arranged in linear as well as branched sequences. Sugars typically present in mucins are *N*-acetylglucosamine, *N*-acetylgalactosamine, galactose, fucose, and sialic acids. Characteristically, mucins lack uronic acids (which are found in proteoglycans) and mannose (which is a principal sugar in asparagine-linked oligosaccharides of serum). The sugar chains are attached to the central protein core through *N*-acetylgalactosamine residues via "O-glycosidic" linkage to serine or threonine.

Mucus is cleared continuously by cilia, which extend through the periciliary fluid to hook the mucus layer. The claw-like tips of the cilia engage with the mucus layer and transport the latter toward the nasopharynx [8,9]. Cilia are complex motile structures that extend from the surface of columnar ciliated cells; the number of cilia per cell is approximately 200 with a cell density of 6–8 cilia per μm^2. These hair-like protrusions range in length between 5 and 10 μm and width from 0.1 to 0.3 μm.

A typical cross section of a cilium shows a ring formed by nine pairs of microtubules and two central tubules, i.e., the so-called nine + two pattern. Each doublet contains an A and a B subfibril with an inner and an outer dynein arm (a complex protein with ATPase activity) located on the A subfibril with radial spokes extending toward the central doublet. The ciliary membrane, which is an extension from the cell membrane of the epithelial cell, encloses the microtubules. The motion of the cilia is dependent on the sliding of the outer doublets past one another with the energy provided by adenosine triphosphate (ATP) through dynein ATPase activity.

The coordination of cilia motility is controlled by neural innervation, chemical pacemaking, and hormonal stimulation, and the effects of ions such as calcium and potassium. Cilia beat in a coordinated fashion to achieve the unidirectional propulsion of mucus with the frequency of the ciliary beat, which is dependent upon the environment. Human nasal cilia have been reported to beat, *in vitro*, with an average frequency of 10 Hz. Any rheological abnormalities in the mucus gel, particularly those that alter the elastic properties of mucus, can greatly affect clearance and undermine ciliary activity in the nasal cavity. In such circumstances, the adhesiveness of the mucus layer may allow it to remain in contact with the underlying cell layers and retain any substances introduced into the nasal cavity.

19.3 PHYSIOLOGICAL BARRIERS TO NASAL DRUG DELIVERY

The physiological barriers to drug absorption from the nasal cavity include losses during drug delivery, mucus permeability, epithelial permeability, drug clearance, degradation, health, and the environment. The relative importance of these barriers is different for small and large molecular weight molecules (Table 19.1). These barriers are considered below.

19.3.1 Delivery to the Nasal Cavity

Administration of drug to the therapeutic target site or absorption site in the nasal cavity is the first step in nasal drug delivery. Uniform distribution over the affected area is desirable for the treatment of nasal symptoms, whereas targeting of the favorable absorption sites

TABLE 19.1
Relative Contribution of Physiological Barriers to the Absorption of Drugs via the Nasal Cavity

Barrier	Small Molecules (% loss)	Large Molecules (% loss)
Degradation	0–15	0–5
Clearance[a]	0–30	20–50
Deposition (anterior loss)	10–20	10–20
Health and environment	10–20	10–40
Epithelial permeability[a,b]	0–30	20–50
Mucus layer	<1	<1

Source: Gizurarson, S., *Adv. Drug Deliv. Rev.*, 11, 329, 1993.
[a]Depends on excipients.
[b]Depends on physicochemical characteristics of the drug.

within the nasal passages is the aim for systemic delivery. Targeting the comparatively small inaccessible olfactory region provides a challenge if nose-to-brain delivery is the objective.

Constraints on nasal delivery include a restricted capacity (i.e., the volume of formulation that can be delivered is limited), attainment of dose accuracy, and the reproducibility of delivery. The distribution of drug in the nasal cavity is highly dependent on the delivery device, formulation, and administration technique. In turn, the distribution of drug will affect permeability, residence time, and metabolism in the nasal cavity.

19.3.2 Permeability

Systemic drug delivery by the nasal route offers many potential advantages over some other routes, including rapid absorption and high systemic availability. As with most sites of drug absorption, the availability of a drug is affected by the area available for absorption, blood flow, and the intrinsic permeability of the epithelium. Other important considerations include contact time between the drug and the absorption site (Section 19.3.3), metabolism of the drug prior to and during absorption (Section 19.3.4), and pathology of the absorbing tissue (Section 19.6.2).

The total surface area of the nasal cavity is about 150 cm^2, with the area available for absorption enhanced by the convolutions of the turbinates and the presence of microvilli on the surface of the ciliated and unciliated cells of the respiratory epithelium. The arterial supply of the nose is particularly rich in the respiratory epithelium where the Kiesselbach's plexus lies, an area that is rich in numerous capillary loops. The nasal blood flow has been shown to be sensitive to the action of a variety of inhaled compounds, both locally or systemically acting. Clonidine has been shown to decrease the blood flow whereas histamine and phenylephrine have been shown to induce the converse effect. Such direct changes to blood flow are important in determining the rate and extent of drug absorption from the nasal cavity.

The permeability barrier in the respiratory region of the nose includes the mucus layer and the airway cells. Mucus potentially affects drug delivery by acting as a barrier to diffusion. *In vitro* studies have indicated that the presence of mucus has the potential to retard the transport of many compounds, although mechanisms are complex and the impact of mucus is difficult to predict.

In an evaluation of the diffusion of drugs across the gastrointestinal mucus, the most important physicochemical characteristic influencing the diffusion of most species was lipophilicity, whereas molecular size had more influence for larger peptide drugs [10]. Binding of

drugs to the mucin is important for positively charged drugs such as amikacin, gentamicin, and β-lactam antibiotics, which bind electrostatically to the negatively charged components in mucus. When the diffusion of a range of β-lactam or aminoglycoside antibiotics through rat intestinal mucus was evaluated, cephaloridine and gentamicin were found to be significantly bound and the degree of binding was found to be dependent upon the pH and ionic strength of the mucus [11].

Interestingly, the use of mucolytic drugs, which alter the viscoelasticity of mucus, has been shown to increase the absorption of intranasally administered human growth hormone (22 kDa). In contrast, other studies have shown that antibodies (150–970 kDa) are able to diffuse through cervical mucus relatively unimpeded, a finding that suggests that the diffusion barrier to antibodies presented by mucus in the nasal cavity might be relatively minor [12].

Overall, it appears that poorly understood interactions (ionic, osmotic, hydrophobic) between the mucus components and drug compounds are likely to be more important in determining the magnitude of the mucus permeability barrier than the gel structure of the mucus alone. As nasally administered drugs are retained in contact with the nasal mucosa for a relatively short period of time, any retardation of diffusion by mucus might be expected to lead to marked changes in bioavailability, although the barrier presented by mucus is suggested to be proportionately low (see Table 19.1).

The nasal epithelium possesses selective absorption characteristics similar to those of a semipermeable membrane, i.e., it allows a rapid passage of some compounds while preventing the passage of others. The process of transportation across the nasal mucosa involves either passive diffusion, via paracellular or transcellular mechanisms, or occurs via active processes mediated by membrane-bound carriers or membrane-derived vesicles involving endo- or transcytosis.

Hydrophilic drug molecules are predominantly absorbed by paracellular absorption through the tight junctions between adjacent epithelial cells. Diffusion is driven by a concentration gradient along the aqueous pathway of the intercellular space of the cells and restricted by tight junctions at the apical surface of the cells such that this route is limited mainly to small hydrophilic molecules. This route is dependent upon the molecular weight and, although slower than transcellular absorption, can be fast enough to give a high systemic availability for low molecular weight polar compounds (<1000 Da).

The effect of molecular size has been demonstrated in a systematic study of a wide range of drugs with molecular weights varying from 160 to 34,000 Da [13]. The results indicated that nasal absorption decreases exponentially as a function of increasing molecular weight. The rate-limiting molecular weight cut off for absorption from the nasal cavity was found to be 1000 Da compared to 300 Da for the oral route. Similarly, a range of different-sized polyethylene glycols were well absorbed from the nasal route up to molecular weights of 2000 Da, after which permeability enhancers were required to improve bioavailability [14]. A comparison of the nasal absorption of uncharged and cationic dextrans in rabbits found that, as expected, the plasma level of the uncharged dextran decreased as its molecular weight increased. In contrast, the plasma level of the positively charged dextran increased up to a molecular weight of 9000 Da, only decreasing when the molecular weight rose further to 17,200 Da [15]. It was speculated that the increasing molecular weight of the FITC DEAE dextran facilitated an interaction with the negatively charged components of mucus and nasal epithelial cells (e.g., sialic acid residues), which enhanced permeability. This encouraged the view that relatively large molecules could be absorbed.

Lipophilic drug molecules are absorbed across the nasal epithelium by passive transcellular diffusion. For small, unionized molecules, this provides a rapid efficient transport mechanism, often resulting in plasma concentration profiles resembling that of intravenous injection and bioavailabilities of up to 100%.

Certain ionized and hydrophilic molecules that are unable to partition into the hydrophobic environment of the membrane lipids may utilize the facilitated transport mechanisms that are present for ions and small molecules, such as glucose. For example, carrier-mediated transport can be an absorption mechanism for peptides and amino acids. The amino acid L-tyrosine is absorbed through the nasal cavity via a carrier-mediated process, which can be increased by esterification of its carboxyl group [16].

In summary, nasal epithelial intercellular junctions are less restrictive compared to the gastrointestinal tract. Such polar pathways will mainly be responsible for the transport of water-soluble compounds, providing a relatively slow, but significant route which is dependent on the molecular weight of the diffusing species. Secondly, transcellular (lipoidal) pathways permit extremely rapid absorption of lipophilic drugs with a rate dependency based on cell membrane partitioning.

19.3.3 Mucociliary Clearance

The cilia, through coordinated beating, transport the overlying mucus layer and upper surface of the periciliary fluid in a unidirectional manner, whereas the periciliary fluid closest to the cell surface is moved back and forth [8,9]. The rate of mucociliary clearance from the nasal cavity to the nasopharynx is highly variable in different regions and under different environmental conditions. Mucociliary activity is regulated by several factors such as temperature, intracellular Ca^{2+} and cAMP levels, and by extracellular ATP. Particles are transported within the viscoelastic mucus blanket at uniform rates irrespective of their viscosity, size, density, or composition. Even large particles, with diameters up to 500 μm, are expelled from the nasal cavity within 10 to 20 min.

Major determinants of the efficiency of mucociliary clearance are cilia density, periciliary fluid, and composition of mucus. Some drugs and excipients, such as preservatives in drug formulations, may diminish the ciliary movement in the nasal cavity and trachea. A suggested adverse effect of ciliostasis (permanently or momentarily arrest or impairment of ciliary activity) is lower respiratory tract infection as a result of impaired nasal microbiological defense.

19.3.4 Metabolism

The nasal epithelium possesses a defensive enzymatic barrier including the presence of phase I and phase II enzymes [17], plus proteolytic enzymes that provide a formidable barrier to the nasal delivery of drugs. Therefore, although hepatic first-pass metabolism that occurs after oral absorption is avoided, the suggestion made over the years that the nasal mucosa might be largely deficient in enzyme activity is now recognized as misguided.

The influence of enzymatic degradation is obviously dependent on the susceptibility of the drug itself. For example, progesterone and propanolol are absorbed rapidly from the nose into the blood without undergoing degradation, but nafarelin acetate has poor systemic availability, perhaps as a result of enzymatic degradation [18–20]. Drug degradation in the nasal mucosa is important not only when considering the nasal delivery of drugs, but also due to the toxicological implications as a consequence of enzymatic transformation of inhaled environmental pollutants or other volatile chemicals. The phase I cytochrome P-450 enzymes can convert some airborne chemicals to reactive metabolites, which may be involved in forming deoxyribonucleic acid (DNA) adducts, increasing the risk of carcinogenesis in the nasopharynx. 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 [21].

The relatively low systemic availability of peptides from the nasal cavity led to the study of proteolytic activity in both *in vivo* and *in situ* systems [22,23]. The presence of both endo- and exopeptidases was found including aminopeptidases A, B, and N, leucine aminopeptidase, and microsomal aminopeptidase with up to 85% of the enkephalin hydrolysis in nasal mucosa homogenate being due to aminopeptidases. The presence of diaminopeptidase, postprolyl cleaving enzyme, angiotensin-converting enzyme, and endopeptidases has also been indicated [24]. This high enzyme activity may explain why enhancing the lipophilicity of peptidase-labile peptides does not have any great effect on bioavailability from the nasal cavity [18]. The increase in lipophilicity could simply increase the partitioning of the peptides into areas of higher enzyme activity where they are metabolized.

The antibacterial enzyme lysozyme is also found in nasal secretions. Lysozyme is produced by the epithelium and mucus glands where it can attack the cell walls of susceptible micro-organisms, its action being optimal at the slightly acidic microclimate pH. The pH of nasal mucus varies with age, sleep, rest, emotion, infection, and diet. When it is cold, or during rhinitis or sinusitis, the pH tends to be alkaline, which deactivates the lysozyme in mucus and therefore increases the risk of microbial infection. Under normal conditions, the nasal secretions, as indicated earlier, have a pH of 5.5 to 6.5, which is the optimum pH for the activity of lysozyme.

19.3.5 Toxicity

In chronic administration to the nasal cavity, or in the treatment of conditions where normal resistance to injury is impaired, concerns about biocompatibility are pertinent. This is particularly important if the use of absorption enhancers is being considered.

Direct toxic effects such as irritation, inflammation, or increased permeability will bring about symptoms such as the sneeze reflex, nasal discomfort, and hypersecretion with the possibility of underlying pathological changes such as squamous metaplasia, cilia erosion, plasma exudation, epithelial necrosis, inflammatory remodeling, or neutrophil accumulation. Indirect adverse effects can also occur and any alteration to normal nasal homeostasis should be avoided. For example, a reduction in mucociliary clearance can cause rhinitis, sinusitis, and an increased susceptibility to airway infections, and consequently ciliary movement should not be altered by any nasal medication. In the context of absorption enhancers, the rate and extent of recovery of normal nasal epithelial function after nasal administration is a prime consideration.

19.4 STRATEGIES FOR OVERCOMING NASAL DELIVERY BARRIERS

19.4.1 Delivery Devices

Delivery devices have a profound impact on drug deposition, for example the tendency for anterior versus more uniform distribution achieved by nasal sprays and solutions, respectively. The simple presentation as nasal drops is simple, economic, and convenient, but it is likely that more sophisticated presentations will be required for many compounds in development. At present typical delivery devices include solutions, nasal sprays (solutions and suspensions), gels, and powders. While the turbinate region is often regarded as the optimal target site for deposition of drugs for systemic absorption on account of the surface area and permeability of the epithelium, it is also a region of rapid mucociliary clearance. Hence the greater anterior deposition resulting from nasal sprays actually results in greater bioavailability on account of the extended residence time.

Current innovations in delivery devices are aimed at improving dose precision, avoiding the requirement for potentially toxic preservatives, enhancing ergonomy and patient usability, and incorporating specialized microsphere/polymer formulations [25].

19.4.2 Molecular Modification

Molecular modification has been advocated as a method for overcoming the barriers posed by the nasal mucosal lining and the enzymatic activity of the nasal epithelium [26]. Two main strategies have been outlined: (i) minimizing degradation through chemical derivitization or covalent attachment to a polymeric carrier and (ii) absorption enhancement through prodrug design or modification of the active molecule.

Classical prodrug strategies such as readily hydrolysable ester compounds have been tested experimentally with some success. For example, the enhanced absorption of ester prodrugs of acyclovir has been reported following modifications that conferred both resistance to degradation and an increased lipophilicity to the parent compound [27]. A limitation of this approach was the level of carboxyesterase activity present in the nasal mucosa (higher than that of the lungs), which resulted in rapid presystemic cleavage of the prodrug ester linkage before the enhanced absorption effect could occur.

In the modification of peptide such as enkephalins for nasal delivery, it is not surprising that the protection of the N-terminal group from the action of aminopeptidases is of prime importance considering the abundance of these peptidases in the nasal epithelium. Protection from C-terminal degradation and an increased lipophilicity are also valuable strategies if these can be achieved without extensively sacrificing aqueous solubility or unduly increasing molecular size or conformational rigidity.

19.4.3 Formulation Approaches

As a result of the physiological constraints outlined, a number of formulation strategies have been devised to optimize or improve nasal delivery of drugs (Figure 19.3).

Bioadhesive formulations and microsphere delivery systems in particular have attracted much attention. As drug formulations are usually rapidly removed from the site of deposition by the mucociliary clearance, increasing the retention time of drug in the nasal cavity via bioadhesion can increase bioavailability [28]. Bioadhesion may be defined as the ability of a material (synthetic or biological) to adhere to a biological tissue for an extended period of time. When applied to a mucous membrane, a bioadhesive polymer may adhere primarily to the mucus layer or epithelial cell surface in a phenomenon known as mucoadhesion [29,30]. The bioadhesive properties of a wide range of materials have been evaluated over the last decade.

Theoretically, bioadhesive dosage forms can provide a platform for sustained/optimized drug delivery that can: (i) localize drug at a specific site for absorption, thereby enhancing bioavailability, (ii) produce intimate contact between the dosage form and the mucosa, improving absorption and allowing for localized modification of tissue permeability, and (iii) prolong residence time, resulting in a delivery system that improves bioavailability or achieves sustained release dosing.

Powdered bioadhesive formulations have attracted much interest for their ability to overcome mucociliary clearance in the nasal cavity. For example, powder formulations of freeze-dried mixtures of insulin and excipients such as crystalline cellulose, hydroxypropyl cellulose, or carbopol 934 have been shown to enhance the absorption of insulin in dogs from the nasal cavity compared to freeze-dried mixtures of insulin and lactose as an excipient [28,31]. The improved delivery was attributed to both the improved dispersion of insulin and the high

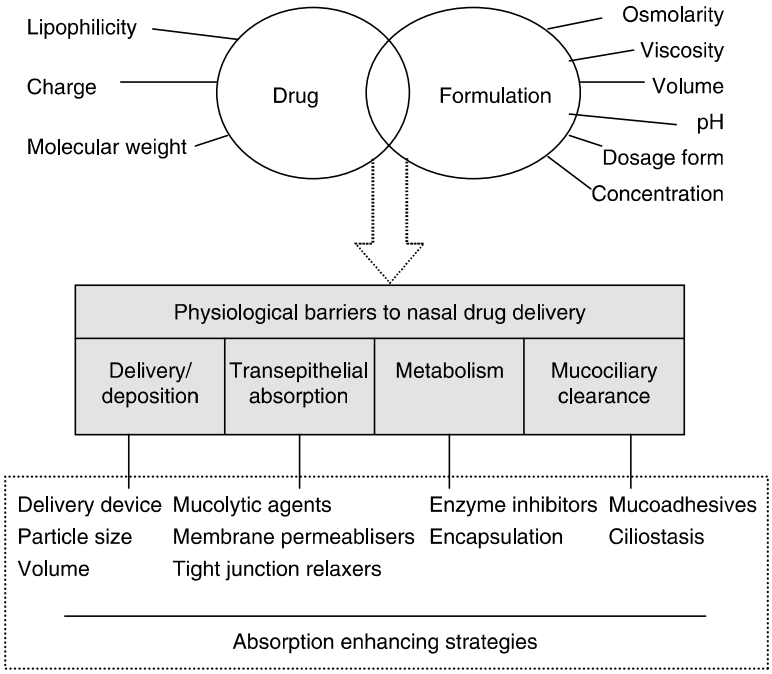


FIGURE 19.3 Drugs for administration via the nasal route have specific formulation requirements which, depending on their ability to overcome physiological barriers, may require an absorption-enhancing strategy.

viscosity of the formulation impairing mucociliary clearance. Similarly, the addition of methyl cellulose to nasal formulations also increases retention time [32]. A bioadhesive liposome formulation has also been shown to provide a therapeutic concentration of nifedipine for prolonged periods of time when delivered via the nose [33]. The administration of the liposomal formulations produced a rapid increase in plasma levels of the drug for an initial 1 to 2 h period followed by a constant plasma level for another 8 to 10 h. In comparison, a simple nifedipine solution achieved a high plasma level followed by a rapid decline.

Mucoadhesive microspheres of hyaluronic acid and chitosan for nasal delivery have been prepared and tested for the delivery of a model drug, gentamicin [34–36]. Hyaluronic acid and mixed hyaluronic acid–chitosan microspheres prolonged the absorption and increased the bioavailability of gentamicin *in vivo* compared to a control solution of the drug. This enhancement was attributed to the bioadhesiveness of hyaluronic acid and the combined bioadhesive and penetration enhancing properties of chitosan. When microsphere formulations were prepared using a combination of the polymers, gentamicin bioavailability increased by twofold compared to administration of the hyaluronic acid formulation alone and 40-fold compared to administration of a solution of the drug.

19.4.4 Absorption Enhancers

Penetration enhancers have been used to facilitate the absorption of higher molecular weight molecules. The mode of action of the surfactant enhancers is often attributed to membrane damage [37]. However, studies in epithelial cell monolayers suggest that some surfactant-based absorption enhancers act primarily by increasing the permeability of tight junctions [38]. Nevertheless, except for the chelators and nonsurfactants, which exert their

influence almost entirely by making the tight junctions between cells more accessible, most penetration enhancers act by increasing membrane fluidity and disturbing the integrity of the membrane to some extent. Thus a concern is that long-term use of enhancers will cause permanent damage to cell membranes and cilia, especially if used in a formulation to treat chronic diseases.

Another type of absorption enhancer, which has been shown to have a better safety profile, is cyclodextrin (CD) [39]. CDs have been shown to form inclusion complexes with lipophilic drugs, thereby improving their aqueous solubility and stability. A powdered insulin formulation containing dimethyl-β-cyclodextrin improved the absolute bioavailability of insulin by 13% in rabbits compared to a control liquid formulation (1%) of insulin with dimethyl-β-cyclodextrin [40]. Recently, hydroxypropyl β-cyclodextrin has been shown to be more effective for enhancing the nasal absorption of acyclovir than a range of other absorption enhancers *in vivo* [41].

Enzyme inhibitors can reduce the metabolic barrier to nasal delivery. The selection of an inhibitor is made on the basis of its ability to inhibit effectively the enzyme primarily responsible for the degradation of a particular compound. The coadministration of peptidase and protease inhibitors such as bacitracin, bestatin, amastatin, and aminoboronic acid derivatives has been found to promote the absorption of LHRH and growth hormone [42,43]. Aminopeptidase inhibitors in particular are effective in improving the bioavailability of enkephalins [44].

19.5 EXPERIMENTAL MODELS OF PHYSIOLOGICAL BARRIERS TO NASAL DRUG ABSORPTION

19.5.1 Nasal Epithelial Models

Drug permeability, metabolism, and toxicity can be evaluated *in vitro* using models of the nasal epithelial in preparation for *in vivo* experiments. Human nasal epithelial cell cultures and animal nasal mucosa mounted in Ussing chambers provide convenient, simple systems in which drug targeting and absorption mechanisms can be investigated under defined, controlled conditions [45].

Primary culture of human nasal epithelial cells can provide suitable epithelial models for the study of drug permeability and metabolism. Healthy human cells from the nonolfactory respiratory region are used to establish the cultures with cells obtained from surgical samples after medical procedures or postmortem biopsies. Nontraumatic procedures such as nasal brushings/scrapings can also be used to harvest cells and have the advantage of being repeatable, although limited numbers of cells are obtained. After harvesting it is necessary to culture the cells to form confluent layers of nasal epithelial cells, using optimized culture conditions to ensure maximal differentiation and the formation of appropriate barrier properties to model the nasal respiratory epithelium. Limitations of primary cultures include the restricted source of cells, interculture variation, and the necessity to establish and verify optimal culture conditions according to experimental requirements.

There are a number of nasal and lung airway cell lines that can be cultured as epithelial cell layers *in vitro*. The RPMI 2650 cell line is of human nasal septal origin and has been advocated as a suitable cell line for use in permeability and metabolism studies [45]. However, limited formation of barrier function by cell layers limits the usefulness of this cell line for drug transport applications. The 16HBE14o- and Calu-3 cell lines of human tracheobronchial origin can also be used to model the nasal epithelium on the basis of the similarity of the airway epithelium in lung and the nose in terms of cell type and morphology. Both these cell lines form suitable cell layers for the investigation of drug delivery applications and are

finding increased use for these purposes [46]. It must be remembered, however, that cell lines do not provide a physiological representation of the mixed cell population of the nasal epithelium and, although there are advantages for studying the permeability in cell layers with tight intercellular barrier properties, the nasal epithelium is less restrictive.

Nasal tissue from animals can be mounted in Ussing chambers, permitting experiments similar to those performed in cell cultures to be performed in intact tissues. The nasal tissues of rabbits, dog, sheep, and cattle have been used and such experiments provide the reassurance that the *ex vivo* system is representative of the nasal mucosa *in vivo*. The limitations of this technique are the requirement for the use of fresh tissue, the limited duration of tissue viability, and interspecies variation in tissue permeability and metabolic capacity.

19.5.2 Mucociliary Clearance Measurement

It is possible to study cilia beat frequency and drug interaction with mucus independently using models such as tissue explants or cultures of ciliated cells or purified mucus preparations. However, these are not able to provide information about mucociliary transport rates. For such studies, a model incorporating the integrated cilia and mucus components is required such as the frog palate. Drugs, preservatives and absorption enhancers, and bioadhesive formulations have been extensively studied in this model [9].

The excised frog palate model is a simple preparation and the most commonly used model to investigate the role of mucus rheology on mucociliary transport. The mucosa is similar to mammalian mucosa with regard to histology, morphology, and function (Figure 19.4). Nasal drug formulations and their components can be evaluated by direct application to the mucus layer of this model (mucus nondepleted). Alternatively, the effect of nasal gels and rheologically modified mucus can be studied by the application to the mucosa after removal of the native mucus (mucus-depleted model). Extrapolation of mucociliary transport rates to clearance *in vivo* is complicated by large intra- and interindividual variations in clearance rates, methodological differences, and higher sensitivity of the *in vitro* technique. Thus, the major

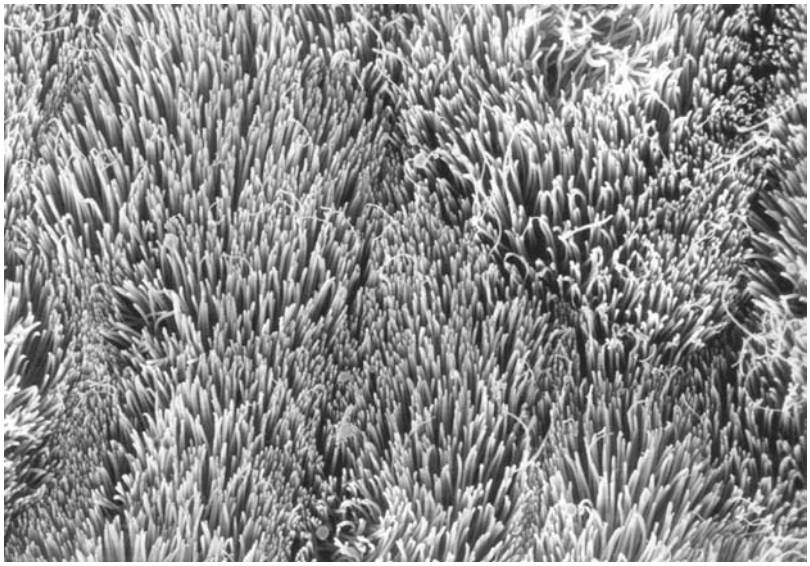


FIGURE 19.4 Scanning electron micrograph of the mucus-depleted ciliated frog palate. The cilia appear in clumps corresponding to the shape of the epithelial cells. (Micrograph provided by Kelly Pritchard.)

application of the technique is screening compounds and formulations for effects on mucociliary mechanisms, rather than prediction of effects *in vivo*.

Mucociliary clearance can be studied *in vivo* in humans using gamma scintigraphy to follow the clearance of radiolabeled solutions from the nasal cavity or by measuring the transport rate of radiolabeled markers administered to the nasal epithelium. Simpler methods are used to monitor the appearance of a strongly colored dye (by visual inspection) or sweet tasting substance such as saccharin (by taste) at the pharyngeal cavity.

19.5.3 *In Situ* Perfused Nasal Cavity

The *in situ* perfused nasal cavity model allows the study of absorption from the intact nasal cavity [47]. In this preparation, drug-containing fluid is introduced into the nasal cavity at the nasopharynx and exits through the nostrils. The fluid may then be recirculated or the model can be operated as a single pass system depending on experimental requirements and drug disappearance is monitored to establish losses to absorption. The benefit of this model in providing absorption data from the intact nasal cavity must be weighed against the limitations, i.e., that perfusion provides a nonphysiological presentation of the drug, which can falsely prolong residence times and provide enhanced absorption or toxicity. In addition, it is not possible to evaluate the effects of formulations such as powders in this model.

19.5.4 *In Vivo* Models

Ultimately, new nasal delivery systems must be tested using *in vivo* models in which it is possible to perform full pharmacokinetic and pharmacodynamic studies. The selection of an appropriate animal species is important and variation in nasal cavity anatomy, mucosal permeability, and metabolic capacity must be considered. Although rats can be used, rabbits are generally accepted as being the best small animal model as they have larger nasal cavities, facilitating drug administration and have sufficient blood volume to permit repeat sampling. If possible, *in vivo* protocols that require anesthesia should be avoided as many anesthetic agents inhibit mucociliary clearance.

19.6 SPECIAL CONSIDERATIONS IN NASAL DELIVERY

19.6.1 Nose–Brain Delivery

The olfactory region located in the poorly accessible recessed roof of the nasal passages offers the potential for certain compounds to circumvent the blood–brain barrier and enter into the brain [48]. The olfactory sensory cells are in contact with both the nasal cavity and the CNS and this neuronal connection constitutes a direct pathway to the brain. By utilizing this pathway drugs would not only circumvent the blood–brain barrier, but also avoid any hepatic first-pass effect and degradation in the blood compartment, a particularly important issue in the case of peptide drugs.

It has been suggested, with the support of experiments conducted in animals, that the rapid euphoria experienced after nasal administration of cocaine is the result of direct absorption to the CNS. Studies in man have also demonstrated a centrally mediated effect of nasally administered drug that was not seen with intravenous injection [48].

The specialized olfactory mucosa features olfactory sensory neurons, which span the nose–brain barrier. At the epithelial surface nonmotile cilia extend from swellings, which lead via a dendritic extension to the neuronal cell body, from which an extended axon penetrates the

cribriform plate of the skull to synapse in the olfactory bulb. The sensory neurons are supported by sustentacular epithelial cells.

Theoretically, substances can be absorbed via the olfactory route by two different mechanisms. Firstly, endocytotic or pinocytotic uptake followed by transport along the axon of the olfactory sensory cells could lead to drug delivery to the olfactory bulb, followed by diffusion to the rest of the brain. This route of transport is relatively slow (hours) and probably not relevant in terms of drug administration. The second route of transport involves paracellular or transcellular transport across the sustentacular cells to the basolateral side of the olfactory epithelium. Diffusion through the perineural space around the sensory nerve axons as they penetrate the cribriform plate into the subarachnoid space completes the nose to brain absorption. This route enables relatively quick (minutes) absorption to the cerebrospinal fluid of hydrophilic and semilipophilic substances and is considered the most likely route of olfactory drug absorption.

Lipophilic drugs are not candidates for this route on account of their rapid absorption to the systemic circulation from the nasal cavity and favorable properties for penetration of the blood–brain barrier. Rather, it is large molecular weight or polar drugs for diseases such as Parkinson's disease and Alzheimer's disease that are relevant for this route. While the present discussion may suggest a clear possibility of nasal administration and subsequent absorption of drug substance directly to the CSF, clinically relevant drug delivery to the central brain via olfactory absorption remains a challenge. Many questions can be raised regarding the feasibility of "nose to brain" absorption given the transport mechanisms involved. The obstacles in targeting absorption via the olfactory pathway and distribution to the central brain may be too great to achieve therapeutic drug concentrations, but this area obviously warrants further investigation in the future.

19.6.2 Disease

Acute or chronic disease can compound the interindividual variations already discussed that contribute to unpredictable nasal drug delivery. For example, respiratory infection may alter respiratory secretion and a number of conditions can lead to mucociliary dysfunction, both of which can greatly affect the clearance rate of nasally administered drugs. Predicting the effects of disease is not straightforward, for example an excessive production of mucus coupled with a reduced capacity for mucus clearance would concomitantly enhance the mucus barrier to drug absorption but increase residence time. Similarly, inflammation may increase epithelial permeability, while nasal hypersecretion would result in dilution and possible changes in the pH. Such effects could result in either enhanced or reduced efficiency of nasal drug delivery.

Chronic diseases include cystic fibrosis in which nasal mucus is thick and viscous as a result of abnormal chloride transport across the membrane of the epithelial cells, leading to reduced water secretion. Similarly, chronic sinusitis also reduces nasal mucociliary clearance due to an increase in the rheological properties of mucus.

Infection and allergy are acute conditions that can affect the nasal cavity. Bacterial infection stimulates secretory cells to synthesize and discharge mucus at a faster rate and can induce an increase in the number of goblet cells. Certain bacteria such as *Streptococcus pneumoniae* and *Streptococcus aureus* produce toxins that disrupt epithelial cells leading to an increase in permeability and damage to the mucociliary clearance system. Rhinovirus infection promotes plasma exudation and increases interleukin (IL)-8 and gel phase mucin secretion, which may contribute to a progression from watery rhinorrhea to mucoid discharge, with mild neutrophilic infiltration during the common cold. Alterations in mucociliary clearance have been extensively studied in asthma, but less frequently in allergic rhinitis.

19.7 CONCLUSION AND CHALLENGES FOR THE FUTURE

The nasal route is generating increasing interest as a route for the administration of local treatments and a cost-effective and patient-friendly alternative to injection for systemic delivery [49]. The special advantages of nasal delivery make it attractive for (i) crisis treatment where rapid onset of action is desirable (e.g., pain, migraine, panic attacks), (ii) systemic delivery of compounds that at present can only be delivered by injection (peptides/proproteins/vaccination), and (iii) direct targeting of the CNS (polar drugs for the treatment of CNS disorders).

To take full advantage of these opportunities offered by nasal delivery, innovative approaches to overcome the biological barriers to delivery are being developed. Advances in formulation design, nasal delivery systems, penetration enhancers, enzyme inhibitors, and bioadhesive polymers require an understanding of the biological barriers that they seek to overcome. Furthermore, appropriate models in which to evaluate new delivery strategies are required. These should be capable of identifying any toxic effects of formulations or excipients, while avoiding misleading results brought about by poor experimental design or model selection. *In vitro* optimization should be undertaken to explore fully fundamental concepts and optimize formulations prior to *in vivo* testing, thereby improving the chances of success and complying with the ethical principles of replacement, refinement, and reduction of animal experimentation.

The challenge for the future is not to let physiological barriers prevent the realization of the unique opportunities offered by nasal drug delivery for safe and effective drug therapy.

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