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Review

The Nasal Mucociliary Clearance: Relevance to Nasal Drug Delivery

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Mucociliary clearance is an important physiological defense mechanism of the respiratory tract to protect the body against noxious inhaled materials. This process is responsible for the rapid clearance of nasally administered drugs from the nasal cavity to the nasopharynx, thereby interfering with the absorption of drugs following intranasal application. This review describes the mucociliary system and the methods used for its characterization. Examples are given of the effects of drugs and additives on its functioning. Further, possible approaches are presented for increasing the residence time of drugs in the nasal cavity, thereby improving intranasal drug delivery.

KEY WORDS: nasal; mucociliary clearance; ciliary activity; drug delivery.

INTRODUCTION

The human nose provides an easily accessible and effective route for the administration of drugs. The nasal mucosa has a large surface area and a rich vascularity, providing for effective drug absorption. Nasal drug therapy has gained in interest since the discovery of potent peptide and protein drugs in the 1970s. These compounds can not be administered perorally because they are poorly absorbed by the gastrointestinal mucosa and proteolytically degraded in the gastrointestinal tract and during hepatic first-pass metabolism. Many of these drugs have been demonstrated to achieve reasonable systemic bioavailabilities after nasal administration (1,2).

To optimize nasal administration, it is necessary to understand how the nose deals with administered drugs and additives and, on the other hand, how these compounds influence nasal integrity and functioning. In these processes, the nasal mucociliary clearance plays a crucial role.

NASAL MUCOCILIARY CLEARANCE

The nasal cavity is divided into two symmetrical halves by the nasal septum. Each halve ends anteriorly in a nostril, and posteriorly it communicates with the nasopharynx. The surface area is enlarged by three conchae (superior, median, and inferior). Next to the olfactory epithelium at the superior turbinate and adjacent septum, there are different types of epithelia lining the nasal mucosa: stratified squamous, pseudostratified columnar or respiratory, and intermediate types (3,4). The stratified squamous and intermediate types are found mainly in the anterior third of the cavity, whereas respiratory epithelium is found in the posterior two-thirds of the cavity. Respiratory epithelium consists of columnar cells with and without cilia, mucus-containing goblet cells, and basal cells (5). Below the epithelium a thick lamina propria is present. It is composed of a loose mesh of fibroelastic connective tissue containing many blood vessels, nerves, and glands. The glands possess both serous and mucus secretory cells; they release directly onto the surface.

Cilia are hair-like protrusions on the free surface of the epithelial cell. They range in length between 5 and 10 μm and in width from 0.1 to 0.3 μm. The number of cilia per cell is approximately 300 (5). 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 ATP'ase activity) located on the A subfibril with radial spokes extending toward the central doublet. A ciliary membrane, which is an extension from the cell membrane of the epithelial cell, encloses the microtubuli (6). Cilia beat in a coordinated fashion. The frequency of the ciliary beat is highly variable with physiological and experimental conditions. In mammals the average beat frequency of cilia in the respiratory tract is approximately 15–20 Hz (7). Human nasal cilia beat with an average frequency of 10 Hz as measured with an *in vitro* test system (8). The motion of the cilia is dependent on the sliding of microtubuli past one another. The energy for the ciliary movement is provided by ATP through dynein ATP'ase activity.

The respiratory epithelium is covered with a mucus layer. The essential structural element of mucus is glycoprotein, formed from a protein core surrounded by carbohydrate side chains, which account for over 70% (by weight) of the molecule. Glycoproteins are responsible for the gel-like

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structure of the mucus. Still, they comprise only 3% of the mucus composition. The remainder consists of water (90–95%), ions, and proteins such as albumin, lysozymes, enzymes, and immunoglobulins (1).

There are two distinctive layers of mucus present on the ciliated epithelium: a lower or sol layer with low viscosity and a more gellous upper layer. Cilia move in the lower layer, whereas the upper layer is transported by the cilia (9). The cilia dip into the gel layer and, with effective beats, transport the layer to the nasopharynx, where it is swallowed (10). During the recovery stroke the cilia move backward through the sol layer, exclusively. The velocity of the mucus transport is ca. 8 mm/min (11). Particles entrapped in the mucus layer are transported with it and, thereby, effectively cleared from the nasal cavity. The combined action of mucus layers and cilia is called *mucociliary clearance*. It is an important nonspecific physiological defense mechanism of the respiratory tract to protect the body against noxious inhaled materials. It should not be hampered by nasally administered drugs and additives, since inhibition of the system results in longer contact times of the nasal mucosa with entrapped viruses and bacteria, possibly leading to infections of the airways. Moreover, a longer contact time of the nasal mucosa with carcinogens, present in air, may result in a higher cancer risk.

On the other hand, the mucociliary clearance is also responsible for the generally observed rapid clearance of nasally administered drugs from the nasal cavity to the nasopharynx. It forms, therefore, an opposing mechanism in the absorption process of drugs following intranasal delivery. In order to investigate the biopharmaceutical aspects and effects of nasal drug formulations on the mucociliary clearance system, methods are needed to characterize its functioning.

ASSESSMENT OF MUCOCILIARY TRANSPORT PARAMETERS

Several methods of assessing the mucociliary function have been described (7,11). They involve determination of its components (the ciliary activity as well as the volume and physical properties of the mucus layer) and those that determine the efficiency of the mucociliary interaction.

Assessment of Mucociliary Clearance

An overview of the methods for measuring the mucociliary clearance in man is given in Table I. Two basically different methods include measurement of the total nasal clearance of a deposited dose and measurement of the transport of markers placed on the mucosa. In the former a radiolabeled solution is deposited in the nose using nose drops or a nose spray. The clearance of radioactivity from the nasal cavity is measured using gamma-scintigraphy. This method gives insight into the overall mucociliary clearance of the nose. It is commonly used for determining the residence time of drug preparations in the nose (12–15). Several approaches serve to determine the nasal mucus transport rate (11,16). In common is the placement of a particle labeled with 99mTc on the ciliary epithelium in the nose. The movement of the particle can be registered using a gamma camera,

Table I. Methods for Measurement of Nasal Mucocillary Clearance in Man

Principle	Detection method	Ref. Nos.
Total clearance of ^{99m}Tc-labeled solutions	Gamma-camera	12–15
Mucus flow rate with ^{99m}Tc-labeled particles	Gamma-camera	16,18–20
Mucus flow rate with radiopaque Teflon disks	Fluoroscope image intensifier	21
Mucocilliary transit time with colouring substances	Pharyngeal inspection	22,24
Mucociliary transit time with saccharin	Sweet taste	16,19,26,27
Mucociliary transit time with a combination of dye and saccharin	Sweet taste and pharyngeal inspection	8,23,25

and the velocity of the particle is subsequently calculated. The method has been modified by decreasing the size of the particle to prevent impairment of ciliary activity and by the use of lower activities (18–20). A serious drawback of gamma-scintigraphic experiments is the administration of radioactive material to volunteers. Sackner (21) blew radiopaque Teflon disks into the nose. The velocity of transport was computed from a roentgenographic image.

The use of dyes to estimate the mucus flow *in vivo* has the advantage of being simple and inexpensive (23–25). A droplet or a particle loaded with a strong dye such as indigo carmine is placed in the anterior part of the nasal cavity, so that the dye will be subsequently transported to the nasopharynx. The time between the placement of the dye and its appearance in the pharyngeal cavity is measured. Repeated inspections of the pharyngeal cavity are necessary, which is inconvenient for the volunteers. An alternative is the deposition of a sweet-tasting substance, such as saccharin (19). In this approach the appearance of the sweet taste is taken as the mucociliary transit time. The determination of the transit time may be influenced by the taste treshold of the subject. A combination of a dye and a sweet-tasting substance will eliminate the disadvantages of both methods (25).

The methods used for measurement of the mucociliary transport rate can be divided into two categories: those in which the tracer material is insoluble (e.g., particle, inert dyes, Teflon disks) and those in which the tracer is soluble (dye solutions, saccharin test). In view of the bilayered structure of mucus, these two methods are quite different. The mucociliary transport rate measured with insoluble particles will reflect only the transport rate of the outer mucus or gel layer. Soluble tracers, however, will dissolve in both layers and may reflect the transport of both the periciliary layer and the gel layer (26). Under normal conditions both layers probably move proportionally and simultaneously in the direction of the beating cilia. This relationship is supported by an inverse correlation as observed between particle transport rate and saccharin transit time in the nose of healthy volunteers (16,26). In some instances, however, the movement of the two layers may be dissociated, resulting in

different transit time values obtained with the two different methods (15,26).

Histograms of mucus transport times in volunteers as obtained with a saccharin/dye test are shown in Fig. 1. Normal mucociliary transit time has been reported to be 12 to 15 min (11), while a mucociliary transit time exceeding 30 min is considered abnormal.

Assessment of Rheological Properties of Mucus

Methods used to evaluate the rheological or flow properties of mucus have been reviewed by Wanner (7) and Marriott (28). As all gels, mucus is a viscoelastic substance possessing both viscous (fluid-like) and elastic (solid-like) characteristics. The viscosity of mucus can be determined with viscosimeters, while the creep compliance and oscillatory test give information on both viscosity and elasticity (28). The excised frog palate represents a model for ciliated epithelium, on which the mucociliary transport of mucus samples can be measured. It relates rheological properties of the mucus directly to effects on mucociliary transport (29,30). Changes in elasticity and viscosity affect the transport rate of mucus on the depleted frog palate (31,32). There is a sharp increase in transport rate with increasing elasticity and viscosity of human ear mucus from patients suffering otitis media up to a maximum value; at higher values the transport rate decreases. The values for normal nasal mucus exist in these optimal ranges (33,34).

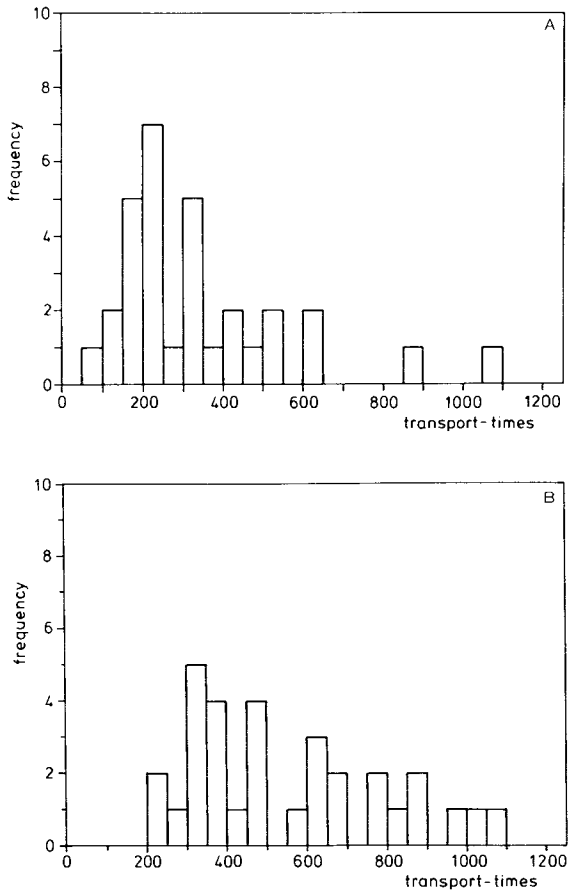

Fig. 1. Histogram of the mucociliary transport time as measured for (A) saccharin sodium and (B) indigo carmine in 31 volunteers. Interval width is 50 sec. Data from Duchateau *et al.* (8).

Assessment of Ciliary Activity

Several methods have been described to measure the beat frequency of moving cilia from the upper airways (7,35) (Table II). Ciliary activity has been assessed by measuring variations in light reflected from moving mucus both *in vivo* and *in vitro* (36,37). Backscattered laser light has been used to determine the frequency of the ciliary beat. The frequency was calculated from the Doppler shift of backscattered photons from the cilia (38,39). The beat frequency of cilia has also been monitored with high-speed cinematography. A motion picture was recorded at high speed and thereafter projected at low speed (40,41). A widely applied method for measuring ciliary activity is the transmitted light technique (42). In principle, light is transmitted through ciliated epithelium, and changes in light intensity due to ciliary movements are detected by a photosensitive cell. The transmitted light technique allows a precise and direct measurement of the beat frequency, and an average value of a group of beating cilia is obtained. The number of cilia measured depends on the system and the cilia samples used and varies between 1 and 2000 (35,43–46). The actual ciliary beat frequency as measured with this device is averaged over a relatively long period of time, during which fluctuations in beat frequency may appear. Fast Fourier transform analysis of the analog signal gives a power spectrum of the fluctuating frequency in this time period (44).

Although some studies report measurement of *in vivo* tracheal ciliary activity or *in vivo* ciliary activity in the paranasal sinuses in rodents (37,39), to our knowledge it is not possible to establish the nasal ciliary beat *in vivo*. Findings from studies which try to relate the *in vitro* nasal ciliary beat frequency with the *in vivo* nasal mucus transport time are rather controversial. Some studies report a good correlation (9,47), while others reject any relation (48,49). These discrepancies may be accounted for by methodologic differences and/or intraindividual variation in mucus transport rate. Nevertheless, it should be emphasized that mucociliary clearance is accomplished by a complex series of interactions among mucus, cilia, and the intervening periciliary fluid. Changes in mucociliary clearance can, therefore, result both from impairment of the ciliary beating (be it frequency, amplitude, coordination, or absolute number of cilia and ciliated cells) and from abnormalities in mucus or periciliary fluid (e.g., viscoelasticity, stickiness, or volume). Consequently, evaluation of mucus properties or ciliary beat frequency *in vitro* cannot always be simply extrapolated to nasal clearance *in vivo;* the *in vitro* approaches provide a good screening method for mucociliary functioning but do not necessarily correlate with nasal clearance *in vivo*.

Ciliary beat frequency measurements have been undertaken to investigate the influence of pH and ionic strength on nasal mucociliary functioning (35,50). Optimal ciliary beat frequency was observed for pH values between 7 and 10. Values lower than pH 6 and higher or equal to pH 11 resulted in severe decreases in beat frequency of chicken embryo trachea. In an isotonic (0.9%, w/v) NaCl solution the ciliary beat is best preserved (Fig. 2). These limits in pH values and

Table II. Measurement of Ciliary Movement

Method	Principle	*Vitro/Vivo*	Tissue	Ref. Nos.
Photoelectric	Changes in light reflections by mucus waves	*Vitro/vivo*	Trachea Paranasal sinus	36,37
Laser light	Doppler shift of backscattered laser light from cilia	*Vitro/vivo*	Trachea	38,39
High-speed cinematography	Recording of a motion picture	*Vitro/vivo*	Frog palate Trachea Nasal respiratory epithelium	40,41
Transmitted light	Intensity variation of light transmitted through cilia	*Vitro*	Trachea Nasal respiratory epithelium	35,43–46

ionic strength may be indicative of the actual physiological properties of the periciliary environment.

INFLUENCE OF DRUGS AND ADDITIVES ON THE MUCOCILIARY SYSTEM

Many drugs and additives appear to have a harmful effect on the nasal, tracheal, and lung mucociliary system (8,51–55). Ideally, drugs and additives in nasal preparations should not interfere with the self-cleaning capacity of the nose. Before they are used in therapy, nasal formulations should, therefore, be assessed for their mucociliary activity (51).

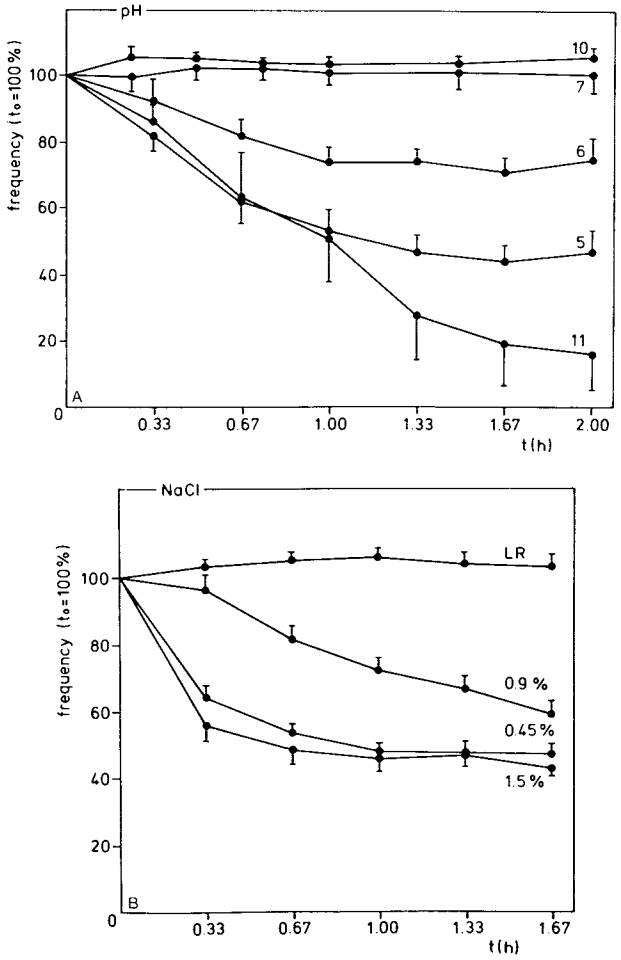

Fig. 2. Influence of pH and ionic strength on the beat frequency of chicken embryo tracheal cilia. (A) Frequency at different pH values; (B) frequency at different concentrations of NaCl and blank Locke Ringer solution (LR). SE is indicated. Data from van de Donk *et al.* (35).

Some nasal drug preparations for local use have been found to inhibit the ciliary beat in chicken embryo tracheas, as well as the nasal mucociliary transport rate in man (25,56). *In vitro* experiments by van de Donk *et al.* showed that mercuric containing preservatives are extremely ciliostatic in an irreversible way. Lipophilic preservatives such as chlorbutol are also ciliostatic, but these effects are reversible. Polar preservatives such as benzalkonium chloride and EDTA appear to be the least harmful (57). It should be noted, however, that some of the observed effects of preservatives on the ciliary beat frequency are different from those found on the transport rate of mucus on the ciliated epithelium in the frog palate model (58,59).

In vitro effects on the ciliary beat may be more pronounced than influences on ciliary activity *in vivo*. During *in vitro* ciliary beat frequency measurements the ciliated tissue is directly exposed to the compounds investigated, whereas in the *in vivo* approaches the cilia are partly protected by the mucus layer. Moreover, under *in vivo* conditions the administered compounds will be diluted by the mucus and eliminated by nasal clearance. The epithelial cells in the nasal mucosa are constantly replaced by cells that differentiate from basal stem cells at the basement membrane. This process may reverse the harmful effects of agents *in vivo*. The possible effect of these agents on mucociliary clearance may, therefore, be dependent on the duration and frequency of contact with the nasal mucosa and the turnover rate of ciliated cells.

An alternative approach, which may come closer to the actual situation of therapeutic application, is the use of an *ex vivo* method for assessing the ciliary movement: the nasal formulation is administered *in vivo*, and after a specified contact time, the ciliary beat frequency of ciliated epithelium is subsequently monitored *in vitro* (60).

The absorption efficiency of poorly permeating drugs (e.g., peptides and proteins) across the nasal mucosa can be improved by the use of absorption enhancers such as bile salts, laureth-9, and fusidate derivatives (61–65). Sodium taurodihydrofusidate, for example, at a concentration of 1% (w/v), is a potent enhancer of intranasally administered insulin in rats, rabbits, and sheep (65,66). However, measured *in vitro* on human ciliated adenoid tissue with the photoelectric method, sodium taurodihydrofusidate has been found to

induce ciliostasis at concentrations of 0.3% (w/v) and higher. This may imply that the enhancer is not suitable for use in nasal insulin therapy. Sodium taurodihydrofusidate is less ciliostatic than laureth-9 (0.3%) or deoxycholate (0.3%), whereas glycocholate and taurocholate exert only a mild effect on ciliary activity *in vitro* (67). Bile salts have been demonstrated to reduce the viscosity of human bronchial mucus (68).

The nasal absorption of many lipophilic drugs is limited because of their poor water solubility. The absorption can be improved by adding solubilizers to the nasal formulation (69,70). Nasal formulations of the lipophilic steroid hormones estradiol and progesterone with dimethyl-β-cyclodextrin as solubilizer resulted in animal experiments in bioavailabilities of 60 and 70%, respectively, as compared to only 20% for control suspension preparations (71,72). Cyclodextrins are biocompatible compounds which are able to form inclusion complexes with lipophilic drugs (73). By this mechanism dimethyl-β-cyclodextrin forms aqueous solutions of steroid hormones (74). These dimethyl-β-cyclodextrin-hormone formulations exert only minor effects on the ciliary beat frequency of human adenoid tissue (Fig. 3), indicating that they have potential for the nasal therapy of estradiol and progesterone (72,75). In addition, dimethyl-β-cyclodextrin is a very potent enhancer of nasal insulin absorption in rats (76). Dimethyl-β-cyclodextrin at a concentration of 5% gave rise to an absolute bioavailability of nasally administered insulin of about 100% and a concomitant strong hypoglycemic response. Since insulin itself is highly water soluble, solubilization of the drug cannot be the mechanism of absorption enhancement in this situation. It has been suggested that cyclodextrins may extract lipids from the gastrointestinal mucosa, thereby leading to facilitated oral drug absorption (77). Such a mechanism might also be implicated in the enhancing effect of dimethyl-β-cyclodextrin on nasal insulin absorption.

Investigating the effects of drugs and additives on nasal mucociliary functioning is an important topical issue, because of the increasing design of nasal drug formulations for pharmacotherapy. These preparations are often designed for long-term treatment. The effects on the ciliated epithelium may limit patient acceptance of the nasal formulation and, thus, the utility in (sub)chronic nasal drug delivery.

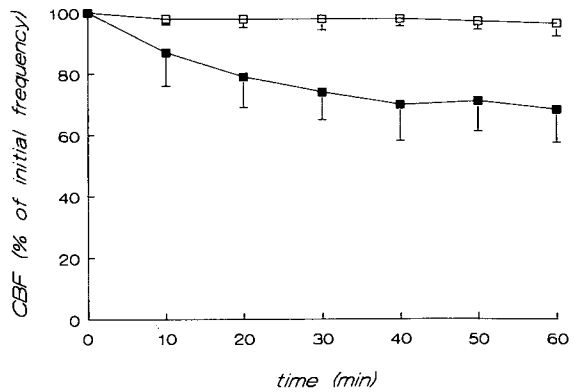

Fig. 3. Time versus ciliary beat frequency plot of human adenoid tissue in (■) progesterone-estradiol dimethyl-β-cyclodextrin solution and (□) blank Locke Ringer solution. Data from Schipper *et al.* (72).

APPROACHES TO IMPROVE THE RESIDENCE TIME OF NASALLY ADMINISTERED DRUGS

The absorption of drugs from the nasal mucosa is influenced by the contact time between drug and epithelial tissue. This contact time is dependent on the clearance of the drug preparation from the nasal cavity. The normal half-life of clearance in man is about 20 min (78,79). A decreased mucociliary clearance might, therefore, affect the absorption profile of nasally administered drugs. Several approaches have been successfully used to reduce the clearance rate. A drug deposited in the nose posteriorly is cleared more rapidly from the nasal cavity to the nasopharynx than a drug deposited anteriorly, because the mucociliary clearance is slower in the anterior part of the nose than the more ciliated posterior part (17). The site of drug deposition in the nose is highly dependent on the dosage form. A large coverage of the nasal epithelial surface will be obtained by application of a large volume from a pipette or a drop bottle. Administration of only few drops will result in a poor distribution over the nasal mucosa (80). Nasal sprays deposite drugs more anteriorly, resulting in a slower clearance of sprays than of drops (12). The nasal bioavailability of the vasopressin analogue desmopressin has been demonstrated to be significantly increased following spray administration as compared to nasal drops (13). Delivery of drugs using a pipette or drop bottle holds the risk that patients, not well instructed, hold the head backward and pour the solution along the nasal floor directly to the rhinopharynx (80). The clearance of a nasal preparation from the nasal cavity may also be influenced by the viscosity of the preparation. Spray preparations containing 0.25% methylcellulose have been reported to exhibit a decreased nasal clearance (81), resulting in a delayed absorption of nasally administered desmopressin, without affecting the bioavailability of desmopressin (82). The clearance half-time of nasal spray solutions containing hydroxypropyl methylcellulose tended to increase with increasing hydroxypropylmethyl cellulose concentration, but the results were not significant (83).

In order to reduce nasal clearance and thereby increase nasal drug absorption, a new concept has been introduced by Illum *et al.* (14), who used albumin, starch, and DEAE-Sephadex microspheres with a diameter of 40–50 μm as nasal dosage forms. These microsphere preparations appeared to have clearance half-life values of 3 hr or more, as compared to 15 min for solutions and powder formulations (Fig. 4). These remarkably reduced clearance times are probably caused by swelling of the microspheres, thereby forming a mucoadhesive intranasal delivery system. Starch microspheres increased the bioavailability of nasally administered insulin and gentamicin in rats and sheep considerably (84–86). Other mucoadhesive delivery systems have also been used for intranasal drug administration. A polyacrylic acid gel base improved the absorption of insulin and calcitonin in rats (87), while cellulose derivatives and neutralized polyacrylic acid (Carbopol 934) increased the nasal absorption of insulin in dogs (88). However, data on the nasal clearances of these dosage forms have not been reported. Recently, a mathematical model has been developed, describing the rate processes involved in nasal drug delivery (89). Using this model the effect of bioadhesive carrier systems can be ac-

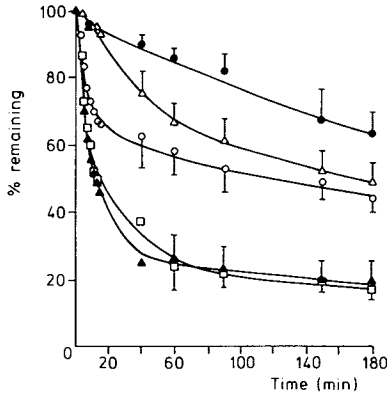

Fig. 4. Clearance of different microsphere systems and of two control systems from the nasal cavity: (●) DEAE-dextran microspheres; (△) starch microspheres; (○) albumin microspheres; (▲) control nasal solution; (□) control nasal powder. Data from Illum *et al.* (14).

curately simulated by reducing the mucociliary clearance rate constant for the transport from the posterior part of the nose to the nasopharynx. The simulations predict that bioadhesion may improve systemic bioavailability and reduce the variability in nasal drug absorption as caused by a variable pattern of drug deposition.

CONCLUSIONS

The applicability of nasal drug delivery depends largely on the effects of the administered drug preparation on the nasal mucociliary clearance, especially if the therapy is aimed for long-term treatment. A number of methods are available to test the effects of drugs and additives on the mucociliary system. These methods involve both *in vivo* and *in vitro* techniques. Although the techniques performed *in vitro* (assessment of ciliary activity, and mucus properties) provide a good screening method, they cannot always be extrapolated to nasal clearance *in vivo*.

In the development of absorption enhancers for the nasal administration of otherwise poorly absorbed drugs, such as peptides and proteins, it is necessary to focus on the effects of the enhancers on nasal mucociliary functioning at an early stage in order to achieve not only efficient but also safe nasal drug therapy.

An increased nasal residence time resulting from a decreased nasal clearance has proven to be an useful approach to improve nasal absorption. In addition, the clearance of nasal drug formulations appears to be dependent on the method of nasal application. A remarkable increase in nasal residence time can be achieved with bioadhesive dosage forms. These formulations have been shown to improve the bioavailability of a number of poorly absorbed drugs. Bioadhesive preparations have promising prospects for the near-future, however, any effects of these preparation on nasal clearance must be reversible, and they have to be tested for long-term effects on mucociliary functioning before use in (sub)chronic nasal drug delivery.

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