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Morphologic examination of rabbit nasal mucosa after nasal administration of degradable starch microspheres

Erik Björk [1,2], Sivert Bjurström [2] and Peter Edman [1,2]

[1] *Department of Pharmaceutics, Biomedical Center, University of Uppsala, S-751 23 Uppsala (Sweden) and* [2] *Kabi Pharmacia Therapeutics AB, S-751 82 Uppsala (Sweden)*

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Summary

Degradable starch microspheres as a dry powder were administered nasally into the left nostril of rabbits, twice per day at 10 or 20 mg per dose. Lomudal[R] Nasal powder (10 mg) was used as a reference. The administrations were run for 2, 4 or 8 weeks. After termination of the experiment, the nasal mucosa was examined by using light microscopy and scanning electron microscopy. The nasal cavity was to a major extent lined by ciliated columnar epithelium. Squamous epithelium was seen on the conchae. A mild hyperplasia of the columnar epithelium on the nasal septum on the left, administered side could be observed in rabbits that had received spheres for 8 weeks. This finding was observed in both doses but was not seen in animals treated for 2 or 4 weeks. No signs of inflammation, erosions or squamous metaplasia were seen. The morphology of the cilia was normal. The results from this study indicate that starch microspheres can be considered to be biocompatible and do not induce serious histopathological changes in the nasal mucosa.

Introduction

Intranasal administration of peptides/proteins has received much attention during recent years (Davis et al., 1986). One obvious reason for this interest is the rapid development of DNA-recombinant technology, which makes it possible to produce peptides/protein for medical use in large quantities. Such drugs are evidently sensitive to the proteolytic environment in the gastro-intestinal tract if they are administered orally. Furthermore, they have a very low bioavailability, and if absorbed, rapid metabolism in the blood and liver will often occur. Consequently, peptides and protein must be administered parenterally. However, the parenteral route, especially intravenous injection, is associated with several drawbacks, rendering it unsuitable for self-medication.

To increase the possibility of self-medication of peptides, an alternative to injection should be sought. In this respect, nasal administration is an attractive alternative. The nasal mucosa is highly vascularized and has a large surface area (Proctor et al., 1976). Proteolytic activity in the nasal mucosa is lower than in the gastro-intestinal tract and permeation is considerably higher.

Although there are several advantages with the nasal route, it still suffers from low drug

Correspondence: E. Björk, Kabi Pharmacia Therapeutics AB, S-751 82 Uppsala, Sweden.

bioavailability. The mechanism by which drugs are absorbed through the mucosa is not well defined. Passive diffusion and paracellular transport are mechanisms which have been suggested (Huang et al., 1985; McMartin et al., 1986). Therefore, the need for absorption enhancers is obvious.

Several experiments have been performed in animals, including humans, with enhancers such as bile acids (Duchateau et al., 1986), fusidic acid derivatives (Longenecker et al., 1987), surfactants of different types and polymers (Hirai et al., 1981a; Morimoto et al., 1985; Raehs et al., 1988). However, long-term use of an enhancer system induces ultrastructural alterations and damage to the nasal mucosa, e.g. necrosis, inflammation or loss of the epithelial layer (Hirai et al., 1981b; Hersey and Jackson, 1987; Daugherty et al., 1988).

A novel nasal drug delivery has recently been proposed by Illum and co-workers (1987) based on degradable starch microspheres. In studies where spheres have been used, with gentamicin in sheep (Illum et al., 1988) and with insulin in rats (Björk and Edman, 1988), a significant improvement in drug bioavailability has been shown.

Because of the damage caused by enhancers, it is natural to raise the question of whether starch microspheres can promote drug absorption through the nasal mucosa without causing mucosal damage.

The purpose of this study was to examine the effect of starch microspheres on nasal mucosa after long-term administration of spheres. The mucosa was examined for potential alterations by using light and scanning electron microscopy.

Materials and Methods

Test material

Degradable starch microspheres (DSM) Spherex^R 45/25 were obtained from Kabi Pharmacia Therapeutics AB, Uppsala, Sweden. Lomudal Nasal 10 mg (Natrii cromoglicas) was purchased from Fisons Pharmaceuticals, Leicestershire, U.K. The microspheres were loaded into gelatin capsules (Capsugel no. 3) to a total amount of 10 and 20 mg, respectively.

Animal experiments

Rabbits of both sexes of the Dutch Cross Breed, weighing 2 kg at the beginning of the study, were used. The animals were shown to be free of clinical respiratory disease on the basis of clinical examination and results of health monitoring. The animals were randomized into four groups and were housed individually (Table 1). Clean cages were provided at least twice a week. Before the experiment the animals were acclimatized to laboratory conditions for at least 2 weeks. The microspheres, 10 or 20 mg, and the nasal powder were administered twice daily into the left nasal cavity using a commercial insufflator (Fisons). The control group only received a puff of air from the insufflator.

The right nasal cavity served as control in all animals and received only a puff of air from the insufflator with an empty capsule. The experiment was run for 2, 4 and 8 weeks.

The body weights were recorded at 2-week intervals and at the end of the experiment.

The animals were deeply anesthetized with pentobarbital i.v. and then euthanized by exsanguination of the femoral arteries. Immediately after exsanguination the nasal cavity was excised.

Light microscopy

The skull was cross-sectioned by cutting the zygomatic arches and the frontal and palatine bones. The nose was then removed in situ without opening the nasal cavity and fixed in 4%

TABLE 1

Experimental design

Week of termination	Number of animals			
	DSM [a]		Lomudal nasal powder [b] 10 mg	Control [b] 0 mg
	10 mg	20 mg		
2	7	7	3	3
4	7	7	3	3
8	7	7	3	3

[a] Five animals were examined by light microscopy and two by scanning electron microscopy.

[b] Two animals were examined by light microscopy and one by scanning electron microscopy.

neutral buffered formaldehyde solution for at least 1 week and then decalcified with a 25% formic acid solution. The nasal cavity was transectioned at two different levels, as shown in Fig. 1. The anterior tissue block was cut 2 cm posterior to the incisor teeth (A) and the posterior block anterior to the first molar teeth (B). Furthermore, specimens from the larynx, the trachea and the cervical lymph nodes were immersed in the same fixative. The tissues were embedded in paraffin, sectioned and stained with haematoxylin-eosin.

Scanning electron microscopy (SEM)

The nasal cavity was opened by removing the nasal and maxillary bones. The cavity was gently flushed with approx. 30 ml of physiological saline in order to remove mucus from the surface. The ventral nasal conchae (Fig. 1) of both sides were cross-sectioned in the middle. The anterior part of the conchae was removed, fixed in Karnowsky's fixative for at least 1 week and postfixed in 1% OsO_4. The specimens were mounted, critical-point-dried, coated with gold-palladium and examined with a JSM 820 scanning electron microscope. All specimens were subjected to an overall examination, followed by photography ($\times 3000$ magnification) of a defined area on the medial surface of the conchae.

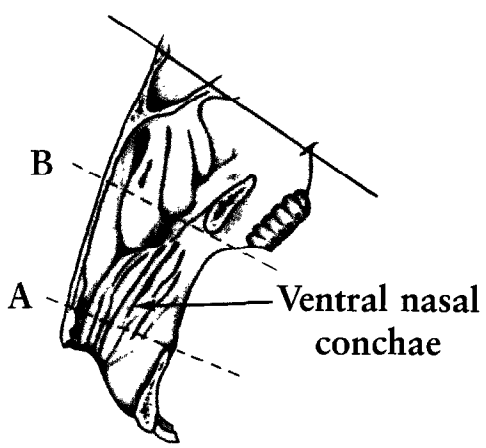

Fig. 1. Schematic drawing of a rabbit's nasal cavity. (A) The level of the anterior cross-section and (B) the posterior cross-section.

Gross examination of the nasal cavity

The animals used for SEM were subjected to a macroscopic examination of the nasal cavity when the conchae were removed. In the animals used for light microscopy the cavity was examined macroscopically after fixation and decalcification when the nasal cavity was cross-sectioned for further histotechnical preparation.

Results

Clinical signs

There were no significant changes in body weights in any of the experimental groups. No clinical signs of respiratory disease, i.e. dyspnea or mucous discharge from the nose, were observed in any of the rabbits.

Gross examination of the nasal cavity

The nasal cavities showed no macroscopic alterations such as changes in architecture, inflammatory reactions or ulcerations.

Light microscopy

In the anterior cross-section, i.e. the region where the vestibule changes into the respiratory area the epithelium was of a stratified nonkeratinized squamous type, most prominent in the ventral part of the cavity and on the conchae. The middle and dorsal parts of the nasal septum were covered with ciliated pseudostratified columnar epithelium. In the posterior section the cavity was lined with ciliated pseudostratified columnar epithelium with numerous goblet cells. The lamina propria contained several seromucous glands and foci of lymphoid tissue.

In some animals given DSM for 8 weeks the administered side of the nasal septum showed mild, focal hyperplasia of the epithelium. The hyperplasia was characterized by a moderate increase in the number of goblet cells and mild hyperplasia of the columnar epithelium with some loss of nuclear polarity. The surface of the hyperplast epithelium was covered with normal microscopic cilia. The hyperplasia was more obvious in animals given 20 mg of DSM than in those given

10 mg of DSM. No such observation could be made in animals given DSM for 4 to 2 weeks or in animals given Lomudal (Fig. 2a and b).

One animal given Lomudal for 4 weeks had mild purulent rhinitis in the left, administered side of the nasal cavity. Erosive rhinitis on the administered side of the nasal septum occurred in one animal given Lomudal for 2 weeks. In all other animals the epithelium was intact without any signs of erosions, inflammatory reactions or squamous metaplasia of the columnar epithelium. The lymphoid tissue in the lamina propria was prominent in some animals, but without any difference between the left and right sides of the

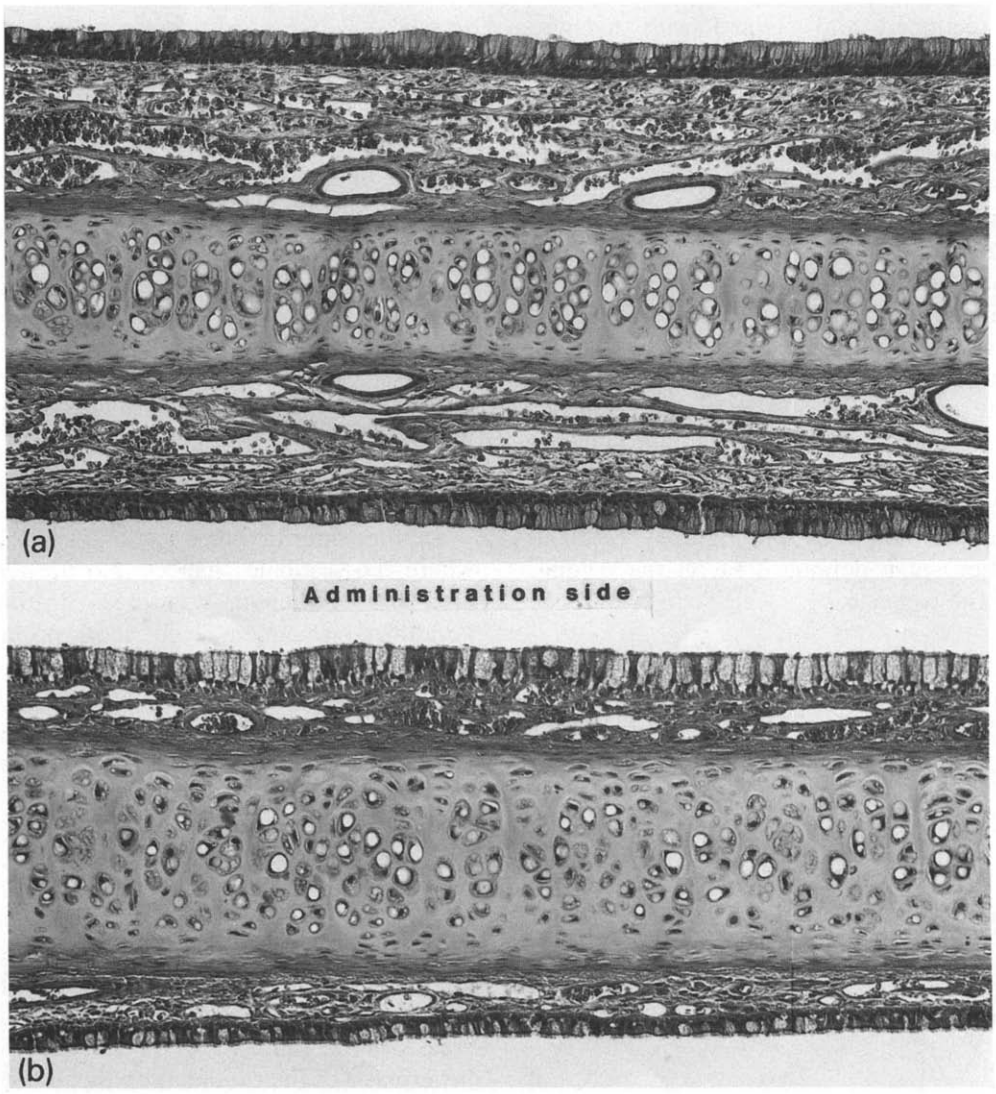

Fig. 2. (a) Cross-section of the septum from one animal in the control group; 8 weeks administration. Columnar epithelium with cilia and goblet cells covers both sides of the septum. LM × 200. (b) Cross-section of the septum from one animal administered DSM, 20 mg for 8 weeks. Mild hyperplasia of the columnar epithelium with an increased number of goblet cells on the administered side. LM × 200.

nasal cavity. The olfactory epithelium could be identified in most animals and showed no signs of atrophy.

Mild acute tracheitis was seen in one animal given Lomudal for 4 weeks. Otherwise the larynx, trachea and cervical lymph nodes were normal and showed no alterations related to the DSM administration.

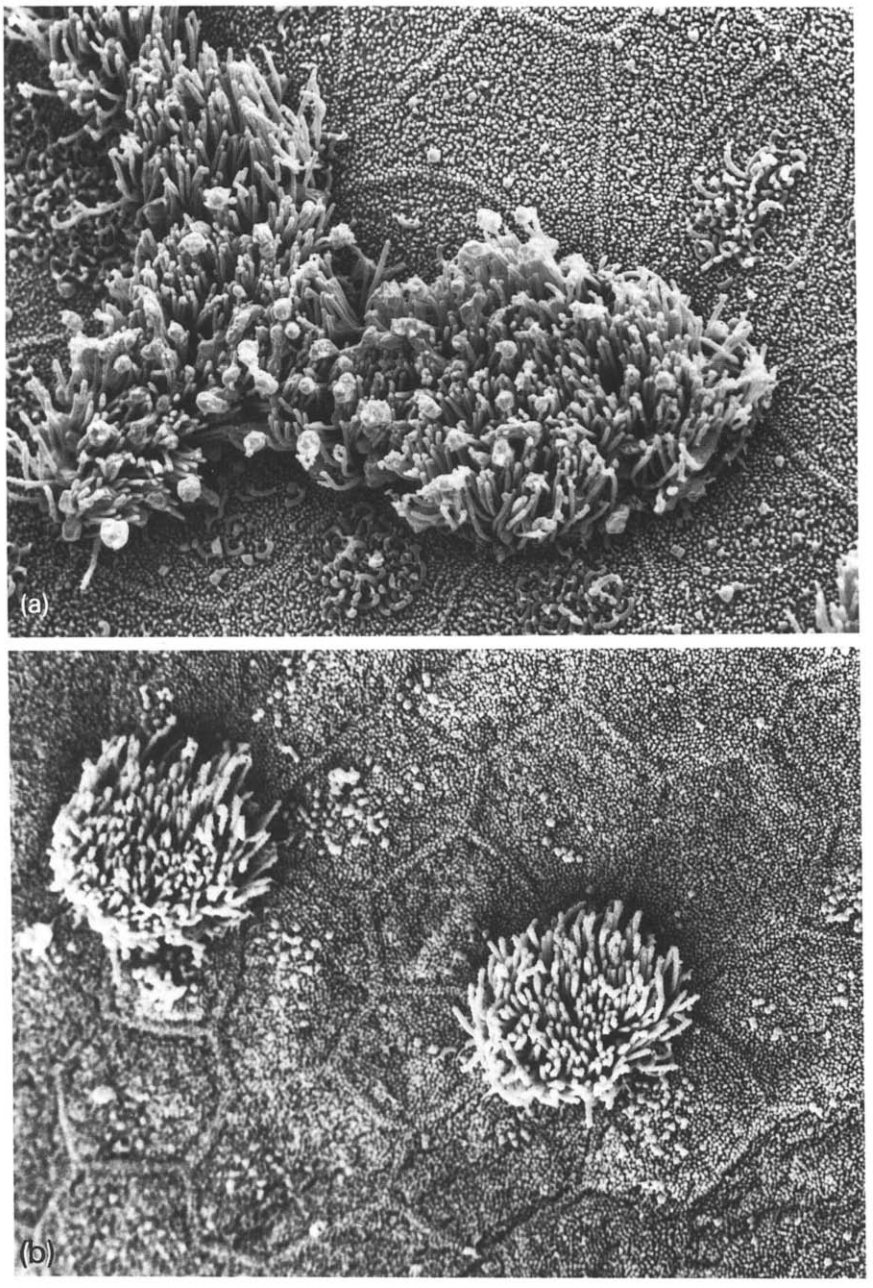

Fig. 3. (a) Ciliated cell from the medial surface of the conchae in one control animal; 8 weeks administration. The knob-like structures at the tips of the cilia were observed both in control and administered animals. SEM × 3000. (b) Ciliated cell from the medial surface of the conchae in one animal administered DSM 20 mg for 8 weeks. SEM × 3000.

SEM

Most of the mucus was removed from the surface, thereby exposing the apical surface of the underlying cells. Squamous epithelium, ciliated cells, goblet cells and non-ciliated cells were easily detected. The anterior part of the ventral conchae specimens was lined with squamous epithelium and solitary ciliated cells. Posteriorly, the number of ciliated cells increased and large portions of the surface were more or less covered by the cilia, most prominently at the bottom of the surface folds. The distribution pattern of ciliated cells was equal between the left and right side, but varied considerably between the animals. The morphology of the cilia showed no variations between the groups (Fig. 3a and b). Foci of a few elevated squamous epithelium cells, regarded as regenerating cells, occurred in all groups.

Discussion

Absorption-promoting substances or systems are widely used in animal studies to increase the systemic uptake of peptides and macromolecules (Longenecker et al., 1987; Deurloo et al., 1989; Igawa et al., 1989). Surface-active agents such as laureth-9 and bile acid derivatives are often used (Aungst et al., 1988), however, they are all associated with toxicological effects on the nasal mucosa impairment of mucociliary clearance (Hermens et al., 1990) and pathological alterations (Hirai et al., 1981a).

Degradable starch microspheres serve as an enhancer system for the hydrophil antibiotic gentamicin (Illum et al., 1988) and insulin (Björk and Edman, 1988).

As the system is administered as a dry powder, it will absorb moisture from the mucous layer and swell. If the spheres are given nasally for a long period it becomes necessary to investigate possible adverse effects on the nasal mucosa.

In this study, the spheres were administered twice daily for up to 8 weeks, 10 or 20 mg spheres per dose and animal. A commercial product, Lomudal Nasal powder, was used as reference. From the SEM pictures it is obvious that the epithelial cells and the nasal cilia remain intact. They have a normal appearance in all animals, irrespective of the type and amount of agent given.

In light microscopy hyperplasia of the epithelial cells on the nasal septum could be seen after the administration of spheres. However, the extent to which this occurred is considered to be small. A plausible explanation of this finding can be that the dose of spheres hit this area immediately after administration, resulting in a secondary hyperplasia. Goblet cell hyperplasia may also be a response to local dehydration, since water is absorbed from the mucosa to the spheres. The protective properties of the mucous layer were unaltered, since no signs of rhinitis occurred in the DSM administered animals. The Lomudal^{R} powder had no visible effect on the nasal mucosa. The difference observed between starch microspheres and Lomudal particles can be explained by the behaviour of the spheres after administration. The starch microspheres take up water from the mucous layer and swell, whereas Lomudal powder dissolves immediately when deposed on the mucosa. This is in agreement with the results obtained when comparing water-soluble starch and water-insoluble starch in the absorption of insulin in rats (Björk and Edman, 1990).

Water-soluble starch did not enhance the absorption of insulin when given intranasally, whereas water-insoluble starch and spheres promoted absorption. This indicates that the mode of action of insoluble starch and starch microspheres involves their ability to absorb water from the mucous layer and probably to affect the epithelial cells in such a way that the paracellular passage of drugs is promoted.

An overall conclusion is that degradable starch microspheres act as a nasal absorption-promoting system for drugs. Further, based on the results from this study, the spheres can be considered to be biologically acceptable with negligible effects on the nasal mucosa.

References

Aungst, B.J., Rogers, N.J. and Shelter, E., Comparison of nasal, rectal, buccal, sublingual and intramuscular insulin

efficacy and the effects of a bile salt absorption promoter. *J. Pharmacol. Exp. Ther.*, 12 (1988) 23–27.

Björk, E. and Edman, P., Degradable starch microspheres as a nasal delivery system for insulin. *Int. J. Pharm.*, 47 (1988) 233–238.

Björk, E. and Edman, P., Characterization of degradable starch microspheres as a nasal delivery system for drugs. *Int. J. Pharm.*, 62 (1990) 187–192.

Daugherty, A.L., Liggitt, H.D., McCabe J.G., Moore, J.A. and Patton, J.S., Absorption of recombinant methionyl-human growth hormone (Met-hGH) from rat nasal mucosa. *Int. J. Pharm.*, 45 (1988) 197–206.

Davis, S.S., Illum, L. and Tomlinson, E., *Delivery Systems for Peptide Drugs*, Plenum, New York, 1986.

Deurloo, M.J.M., Hermens, W.A.J.J., Romeyn, S.G., Verhoef, J.C. and Merkus, W.H.M., Absorption enhancement of intranasally administered insulin by sodium taurodihydrofusidate (STDHF) in rabbits and rats. *Pharm. Res.*, 6 (1989) 853–856.

Duchateau, G.S.M.J.E., Zuidema, J. and Merkus, F.W.H.M., Bile salts and intranasal drug absorption. *Int. J. Pharm.*, 31 (1986) 193–199.

Hermens, W.A.J.J., Hooymans, P.M., Verhoey, J.C. and Merkus, F.W.H.M., Effects of absorption enhancers on human nasal tissue ciliary movement in vitro. *Pharm. Res.*, 7 (1990) 144–146.

Hersey, S.J. and Jackson, R.T., Effect of bile salts on nasal permeability in vitro. *J. Pharm. Sci.*, 76 (1987) 876–879.

Hirai, S., Yashiki, T. and Mima, H., Effect of surfactants on the nasal absorption of insulin in rats. *Int. J. Pharm.*, 9 (1981a) 165–172.

Hirai, S., Yashiki, T. and Mima, H., Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. *Int. J. Pharm.*, 9 (1981b) 173–184.

Huang, C.H., Kimura, R., Bawarchi-Nassar, R. and Hussain, A., Mechanism of nasal absorption of drugs II: Absorption of L-tyrosine and the effect of structural modification on its absorption. *J. Pharm. Sci.*, 74 (1985) 1298–1301.

Igawa, T., Maitani, Y., Machida, Y. and Nagai, T., Effect of absorption promoters in intranasal administration of human fibroblast interferon as a powder dosage form in rabbits. *Chem. Pharm. Bull.*, 37 (1989) 418–421.

Illum L., Jörgensen, H., Biisgard, H., Krogsgaard, O. and Rossin, N., Bioadhesive microspheres as a potential nasal drug delivery system. *Int. J. Pharm.*, 39 (1987) 189–199.

Illum L., Farraj, N.F., Critchley, H. and Davis, S.S., Nasal administration of gentamicin using a novel microsphere delivery system. *Int. J. Pharm.*, 46 (1988) 261–265.

Longenecker, J.P., Moses, A.C., Flier, J.S., Silver, R.D., Carey, M.C. and Dubovi, E.J., Effects of sodium taurodihydrofusidate on nasal absorption of insulin in sheep. *J. Pharm. Sci.*, 76 (1987) 351–355.

McMartin, C., Hutchinson, L.E.F., Hyde, R. and Peters, G.E., Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. *J. Pharm. Sci.*, 76 (1986) 535–540.

Morimoto, K., Morisaka, K. and Kamada, A., Enhancement of nasal absorption of insulin and calcitonin using polyacrylic acid gel. *J. Pharm. Pharmacol.*, 37 (1985) 134–136.

Proctor, D.F. and Adams, K.G., Physiology and pharmacology of nasal function and mucus secretion. *Pharmacol. Ther.*, 2 (1976) 493–509.

Raehs, S.C., Sandow, J., Wirth, K. and Merkle, H.P., The adjuvant effect of bacitracin on nasal absorption of gonadorelin and buserelin in rats. *Pharm. Res.*, 5 (1988) 689–693.