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Toxicological aspects of the use of dextran microspheres and thermogelling ethyl(hydroxyethyl) cellulose (EHEC) as nasal drug delivery systems

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Abstract

The impact on the integrity of nasal mucosa and the ciliary beat frequency after nasal administration of two formulations enhancing absorption of insulin have been evaluated. Dextran microspheres did not influence the ciliary beat frequency in vitro but the ethyl(hydroxyethyl) cellulose (EHEC) system caused irreversible ciliostasis. The hypoosmotic nature of the gel was the major reason for the observed effect. Modest goblet cell hyperplasia was observed in nasal mucosa after repeated administration of dextran microspheres and thermogelling EHEC. The changes observed were located in the anterior part of the nasal cavity. This area is probably exposed to dehydration by the spheres and prolonged hypoosmotic conditions by the EHEC system due to the deposition of the delivery systems in the anterior part of nasal cavity. However, both dextran microspheres and thermogelling EHEC can be considered as relatively safe vehicles for nasal administration of drugs.

Keywords: Nasal administration; Dextran microsphere; Thermogelling polymer; Histology; Ciliotoxicity

1. Introduction

Nasal administration of large hydrophilic drugs, such as peptides and proteins, have attracted great interest during the last 10–15 years. The relatively large surface area, avoidance of first-pass metabolism, rich vascularisation and the ease of self-administration for the patient have been grounds for intense research in this area. Due to the high molecular weight and the hydrophilic characteristics of these drugs, the bioavailability is poor. Therefore a variety of absorption enhancers have been tested. Surfactants (Hirai et al., 1981) and bile salts (Hirai et al., 1978) were the first agents to be tested. Thereafter, a number of promoters have been investigated, such as lysophosphatidylcholine, LPC

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(Illum et al., 1989), sodium taurodihydrofusidate, STDHF (Longenecker et al., 1987), sodium caprate (Mishima et al., 1987), bacitracin (Raehs et al., 1988), degradable starch microspheres, DSM (Bjork and Edman, 1988), cyclodextrins (Merkus et al., 1991) and polymer solutions (Morimoto et al., 1985; Morimoto et al., 1991). All of them were successful in promoting absorption of large hydrophilic peptides and proteins in different animal species. For many of the new interesting peptides and proteins developed by recombinant DNA technique the therapy is chronic or subchronic, so the safety aspect of a new delivery system must be considered. This is usually done by evaluating histopathological changes in the mucosa and determining the effect on the mucociliary clearance. Mucociliary transport and clearance can be measured in vivo (Harris et al., 1986; van de Donk et al., 1982) or in vitro (Gizurarson et al., 1990). Determination of ciliary beat frequency in vitro can be carried out on explants of ciliated mucosa from human adenoid tissue (Hermens et al., 1990), the trachea of different animal species (Jian and Po, 1993; Khan et al., 1986) or the chicken embryo trachea (van de Donk et al., 1980a). Effects on the ciliary beat frequency detected in vitro may be more pronounced than the influence on the ciliary clearance in vivo. In vitro experiments expose ciliated epithelia directly to the test solution, whereas in the in vivo situation the cilia are protected by mucus. Further, a solution given intranasally will be diluted in the mucus, giving lower concentration of potential ciliotoxic agents and, moreover, it will be cleared from the nasal cavity by mucociliary clearance. The turn-over rate of epithelial cells in the respiratory tract is rather fast. The nasal septum of rabbits recovered in 5 days after removal of the mucosa (Ohashi et al., 1991), and removal of an 800-μm long strip of tracheal epithelium in guinea pigs had recovered in 8 h (Erjefält et al., 1994). This may also reduce the damaging effect of a substance in vivo. Therefore, in vitro ciliary beat frequency tests should be used as indicators for potential damage to the nasal epithelium rather than proof of such damage occurring in the in vivo situation.

Absorption enhancers will cause the barrier function to increase transport of otherwise low-permeable drugs. Damaging effects on either epithelial cells, mucus or cilia will increase permeability of the membrane to drug molecules but the barrier function to noxious materials may also be lost. Examination of possible adverse effects of absorption promoters on the nasal epithelium and on the mucociliary clearance must be performed to evaluate the usefulness of such agents. Many of the tested absorption enhancers have a harmful effect on nasal epithelium and on the mucociliary system in the concentrations used to achieve improved absorption. The surfactant laureth-9 and bile salts such as lipophilic sodium deoxycholate and sodium taurodeoxycholate induce severe morphological alterations of the epithelium (Ennis et al., 1990) and inhibit the mucociliary transport in vitro (Gizurarson et al., 1990). The more hydrophilic sodium glycocholate has less effect on both epithelial integrity and CBF (Hermens et al., 1990; Hosoya et al., 1994). Exposure to lysophosphatidylcholine resulted in great alteration in morphology of the nasal epithelium (Chandler et al., 1991; Hosoya et al., 1994), and mucociliary transport in vitro was inhibited (Gizurarson et al., 1990). STDHF has larger impact on the integrity of the nasal epithelium than sodium glycocholate but less effect than laureth-9 and sodium deoxycholate (Hosoya et al., 1994). Administration of degradable starch microspheres twice daily for 8 weeks only induced slight hyperplasia in the mucosal membrane (Bjork et al., 1991), and no changes in mucociliary clearance in vivo could be found after repeated administration (Holmberg et al., 1994). The damaging effect of cyclodextrins depends on the structure of the oligosaccharide. Some of the tested cyclodextrins induced ciliostasis in vitro and disorder in the exposed tissue, whereas others only caused a slight decrease in CBF and had no damaging effect on nasal septum (Gill et al., 1994; Merkus et al., 1991). Histological evaluation of polymer solutions has only been performed after rectal administration of polyacrylic acid aqueous gel. No mucosal damage was observed (Morimoto et al., 1980). The ciliary beat frequency measured in

vitro was not affected by a hyaluronic acid solution (Morimoto et al., 1991).

Two new nasal delivery systems, dry dextran microspheres and thermogelling polymer solution of ethyl(hydroxyethyl) cellulose (EHEC), promote nasal absorption of insulin (Rydén and Edman, 1992). The particle system is based on solid epichlorohydrine cross-linked dextran spheres (Sephadex®). Dextran microspheres of mean particle size 32 μm with insulin distributed on the surface of the spheres gave a larger decrease in plasma glucose than microspheres with insulin incorporated into the spheres (Pereswetoff-Morath and Edman, 1995a). Ethyl(-hydroxyethyl) cellulose has a low critical solution temperature (LCST) of approx. 32°C, i.e. the solubility of the polymer is dependent on the temperature. At temperatures above the LCST, EHEC will precipitate and phase separation will occur. When a small amount of sodium dodecyl sulphate is added to the non-ionic polymer EHEC, the LCST is increased (Carlsson et al., 1986). Instead of phase separation at temperatures close to body temperature, a highly viscous gel is formed. The increase in viscosity is due to the formation of an expanding network that incorporates water. The network can be formed by the sodium dodecyl sulphate forming mixed micelles with the EHEC polymer chains (Carlsson et al., 1989). Therefore, at a temperature of 35–37°C, the system will not separate, and a homogeneous gel is formed instead. A hypoosmotic gel gave a rapid decrease in plasma glucose, whereas isoosmotic and hyperosmotic gels did not affect the glucose level (Pereswetoff-Morath and Edman, 1995b).

Neither of these systems has been tested for damaging effects on the nasal mucosa. The aim of this investigation was to evaluate the safety of dextran microspheres and hypoosmotic thermogelling polymer EHEC as nasal drug delivery systems. The mucosa was examined for potential damage by light and scanning electron microscopy after repeated administration. Ciliotoxicity was determined on rat tracheal rings in vitro.

2. Materials and methods

2.1. Material

Dextran microspheres (Sephadex® G25 Fine) were obtained as a gift from Pharmacia, Uppsala, Sweden. Ethyl(hydroxyethyl) cellulose (EHEC) was obtained from Berol Nobel, Stenungsund, Sweden. Human monocomponent insulin (Actrapid® 100 IU/ml) was purchased from Novo, Denmark. Sodium dodecyl sulphate (SDS), spec. pure, was purchased from BDH, Germany. All other chemicals were of analytical grade. For preparation of solutions Millipore-water (Milli-Q^{UF}) was used.

2.2. Preparation of spheres and solutions for in vivo exposure

Dextran microspheres were sieved and the fraction smaller than 45 μm was used in the experiments. A stock solution of 1% (w/w) EHEC in water with 3 mM SDS was prepared and kept in the refrigerator for one week to ensure complete dissolution (Carlsson et al., 1990). By adding water and Actrapid®, a hypotonic polymer solution of 0.6% EHEC, 1.8 mM SDS, and an insulin activity of 30 IU/ml was prepared. The plain vehicle (0.6% EHEC and 1.8 mM SDS) without insulin could not be tested because the concentration of SDS is too low to form the cross-links needed between the polymer-chains for formation of a gel. Therefore, addition of Actrapid® was necessary to ensure thermal gelation, because both the preservative *m*-cresol in Actrapid® and insulin participate in the gel formation. So, in spite of the low SDS concentration, an increase in viscosity is obtained at elevated temperatures for the complete system (Pereswetoff-Morath and Edman, 1995b). The control solution containing 1.8 mM SDS was made in physiological saline.

2.3. Preparation of spheres and solutions for ciliary beat frequency measurements

The Krebs buffer had the following composition (mM): NaCl 120, KCl 4, $NaHCO_3$ 20, NaH_2PO_4 1.5, $MgSO_4$ 1.5 $CaCl_2$ 1.5 and glucose

10. The pH was 7.4 and the solution was aerated with 5% CO_2 in O_2. The substances tested were dissolved in Hepes buffer consisting of (mM): NaCl 135, KCl 4.6, $MgSO_4$ 1.2, $CaCl_2$ 1.5, glucose 11 and Hepes 10. pH was adjusted to 7.4 with Tris-(hydroxymethyl)-aminomethane. The EHEC gel system consists of 0.6% EHEC, 1.8 mM SDS, 30 IU/ml insulin, 0.9 mg/ml *m*-cresol, and 4.8 mg/ml glycerol. The latter three originate from Actrapid®. The substances in the EHEC gel were also tested individually in Hepes buffer in the same concentrations as in the gel system. Dextran microspheres were sieved and the fraction smaller than 45 μm was applied in a dry state.

2.4. In vivo exposure

Male Sprague–Dawley (Møllegaard, Denmark) initially weighing 200–250 g were used in the experiments. The animals were randomised into nine groups (Table 1) and kept under standardised conditions. Clean cages were provided twice a week and the rats had free access to water and food. Before the experiments the animals were acclimatised to laboratory conditions for 2 weeks. To facilitate dosing, the rats were anaesthetised by inhalation of 4% halothane (Halothane, ISC Chemicals Ltd, England) for 90 s and the dose was delivered during the 30–45 s that the anaesthesia lasted. The anaesthetic was given in 50% O_2 and 50% N_2O at a flow rate of 1 l/min in a vaporiser specially built for small animals. All the preparations were administered with a PE-90 polyethylene tube inserted 5 mm into the right nostril. Dextran spheres (1.25 mg) were weighed into the tube and administered by blowing air from a syringe through the tube. As control, one group received just a puff of air. The solutions, 25 μl, were administered by a Finnpipette, Labsystems. As controls to the EHEC polymer system, water and physiological saline were used as hypotonic and isotonic controls, respectively, and an isotonic 1.8-mM SDS solution was also administered. The rats were administered once daily and the experiment was run for 2 and 4 weeks. All control groups were run for 4 weeks. To exclude influence of the inhalation anaesthesia, one group was just anaesthetised, and one group was neither anaesthetised nor administered intranasally. At termination of the experiments, approx. 12 h after the last administration, the animals were deeply anaesthetised with an intraperitoneal injection of tiobutabarbital sodium (Inactin, BYK) and then euthanised by exsanguination of the femoral arteries.

Table 1
Experimental design for in vivo exposure. <TB1>

Type of intranasal administration	Number of animals[a] in the two groups administered:	
	2 weeks	4 weeks
1% EHEC, 3 mM SDS	5 (2,3)	5 (2,3)
0.6% EHEC, 1.8 mM SDS, and Actrapid®	5 (2,3)	5 (2,3)
Dextran microspheres	5 (2,3)	5 (2,3)
1.8 mM SDS in physiological saline	-	3 (1,2)
Physiological saline	-	3 (1,2)
Water	-	3 (1,2)
Airpuff	-	3 (1,2)
Inhalation anesthesia	-	3 (1,2)
Untreated	-	3 (1,2)

</TB1>[a]In parentheses number of animals for morphological or histological examination, respectively.

2.5. Tissue collection and fixation for light microscopic examination

The animals were decapitated and skin, lower jaw and posterior bone structures of the skull were removed. The heads were fixed in 4% neutral buffered formaldehyde solution for at least 1 week. The nose was decalcified with phosphate-buffered saturated EDTA-solution, pH 7, for at least a week, and cross-sectioned at three different levels (Fig. 1). One anterior section was taken close to the nostrils, one section from the middle part of the nose, mainly involving the respiratory epithelium. The third section was taken from the posterior part of the nasal cavity for examination of the olfactory epithelium. The samples were embedded in paraffin, sectioned, stained with haematoxylin-eosin and examined by light microscopy.

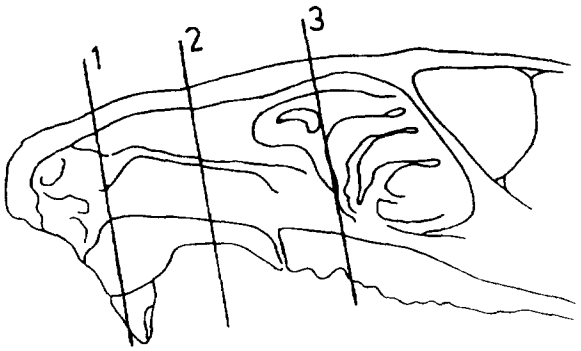

Fig. 1. Schematic drawing of rat nasal cavity. (1) The level of anterior cross-section, (2) the level of middle cross-section, and (3) the level of posterior cross-section.

2.6. Tissue collection and fixation for scanning electron microscopic examination

The nasal cavity was opened by removing the nasal and maxilliary bones. In order to remove mucus the cavity was gently flushed with physiological saline. The nasal septum at the administration side was excised and fixed in Karnowsky's fixative for at least 1 week and then finally fixed in 1% OsO_4. The specimens were mounted, critical point dried, coated with gold-palladium, and examined with a JSM 820 scanning electron microscope (SEM).

2.7. Ciliary beat frequency measurements

Rats weighing approx. 300 g were used in this study. The animals were killed by a blow to the head. The trachea, along with the main bronchi, were removed immediately and placed in aerated Krebs solution. Connective tissue was carefully removed and the trachea was cut into small rings of about 0.3–0.5 mm width with the help of a dissecting microscope. Adhesive mucus was washed off with Krebs solution. The tracheal preparation in a drop of Hepes buffer was transferred to a cover slip, which was placed upside down on a glass slide, 2 mm thick with a 1.2-mm deep cavity in the centre, giving a preparation hanging freely in a drop of Hepes buffer (Khan et al., 1986).

The hanging drop preparation was placed on a microscope stage, maintained at room temperature (20–21°C) in all experiments except for the experiments with gelling EHEC solution that were performed at 37°C. The beating cilia were viewed at $\times 400$ magnification in a Niko-optiphoto (chromatic aberration free) microscope. The cilia were oriented to interrupt the passage of light through a slip in a diaphragm (0.2 mm) into the photometer, Nikon photometer P 1, which transduced the light energy into an electrical signal. The electrical signal generated was converted into a reading of ciliary beat frequency (CBF) displayed on the screen of a Nicolet 3091 oscilloscope. The signals displayed on the oscilloscope were also recorded on paper by means of an X-Y BD 90 recorder. The preparations were allowed to equilibrate for at least 15–20 min. CBF in each preparation was recorded from six different sites and a mean frequency calculated. All CBF measurements were performed this way. The preparation was then immersed into the test solution or completely covered with the dry microspheres (10 mg) and, after 15–30 min exposure, the CBF was again recorded. To study the recovery of the ciliary activity, the test solution was washed out thoroughly with Hepes buffer, allowed to stand for 15 min, and CBF was recorded as described above. The results were expressed as means $\pm$ S.E.M. of six different tracheal rings. Statistical evaluation was performed by means of Student's t-test for paired observations.

Table 2
Number of rats with histopathological findings in the nose after different local treatments

Treatment	Control groups						Test groups					
	None	Anaesthesia	Air puff	Water	Phy. saline	SDS	Dextran microspheres		EHEC (%)			
									0.6		1	
Weeks	4	4	4	4	4	4	2	4	2	4	2	4
No. animals	2	2	2	2	2	2	3	3	3	3	3	3
Nasal cavity, anterior part												
Focal erosion												
-minimal	—	1	—	—	—	—	—	—	—	—	—	—
Distended lacrimal duct												
-mild	—	—	—	1	—	—	—	1	—	—	1	1
Hyperplasia, focal												
-mild-moderate	—	—	—	—	—	1	—	3	—	—	—	1
Squamous metaplasia, focal												
-mild	—	—	—	—	—	—	—	2	—	-	—	—
Nasal cavity, middle part												
Mucosal cyst												
-mild	—	—	—	—	—	—	1	—	—	—	—	—
Erosion, focal												
-mild	—	—	—	—	1	1	1	—	—	—	—	—
Prominent lymphoid tissue												
-mild-moderate	—	2	1	2	2	2	3	2	1	3	2	3
Nasal cavity, posterior part												
Erosion, focal												
-mild	—	—	—	—	—	—	1	—	—	—	—	—
Prominent lymphoid tissue												
-mild	—	1	—	2	1	—	—	1	—	1	1	—

3. Results

3.1. Light microscopic examination

Only minor findings were observed at the histopathological examination, most frequent in the anterior part of the nose (Table 2). The two untreated control rats showed no histopathological changes in the respiratory epithelium at the septal wall (Fig. 2A). All three rats subjected to dextran microspheres for 4 weeks, one out of three rats given EHEC 1% for 4 weeks, and one out of two rats given SDS for 4 weeks, showed minimal to mild focal hyperplasia of the respiratory epithelium of the nasal septum. The hyperplasia was characterised by an increase in the number of goblet cells and hyperplasia of the columnar epithelium with some loss of nuclear polarity. The surface of the hyperplastic epithelium was covered with normal microscopic cilia. Two of the rats given dextran microspheres for 4 weeks also displayed mild focal squamous metaplasia (Fig. 2B), a finding also observed in one rat administered physiological saline intranasally for 4 weeks. Rats subject to administration of 0.6% EHEC with 1.8 mM SDS and Actrapid® showed

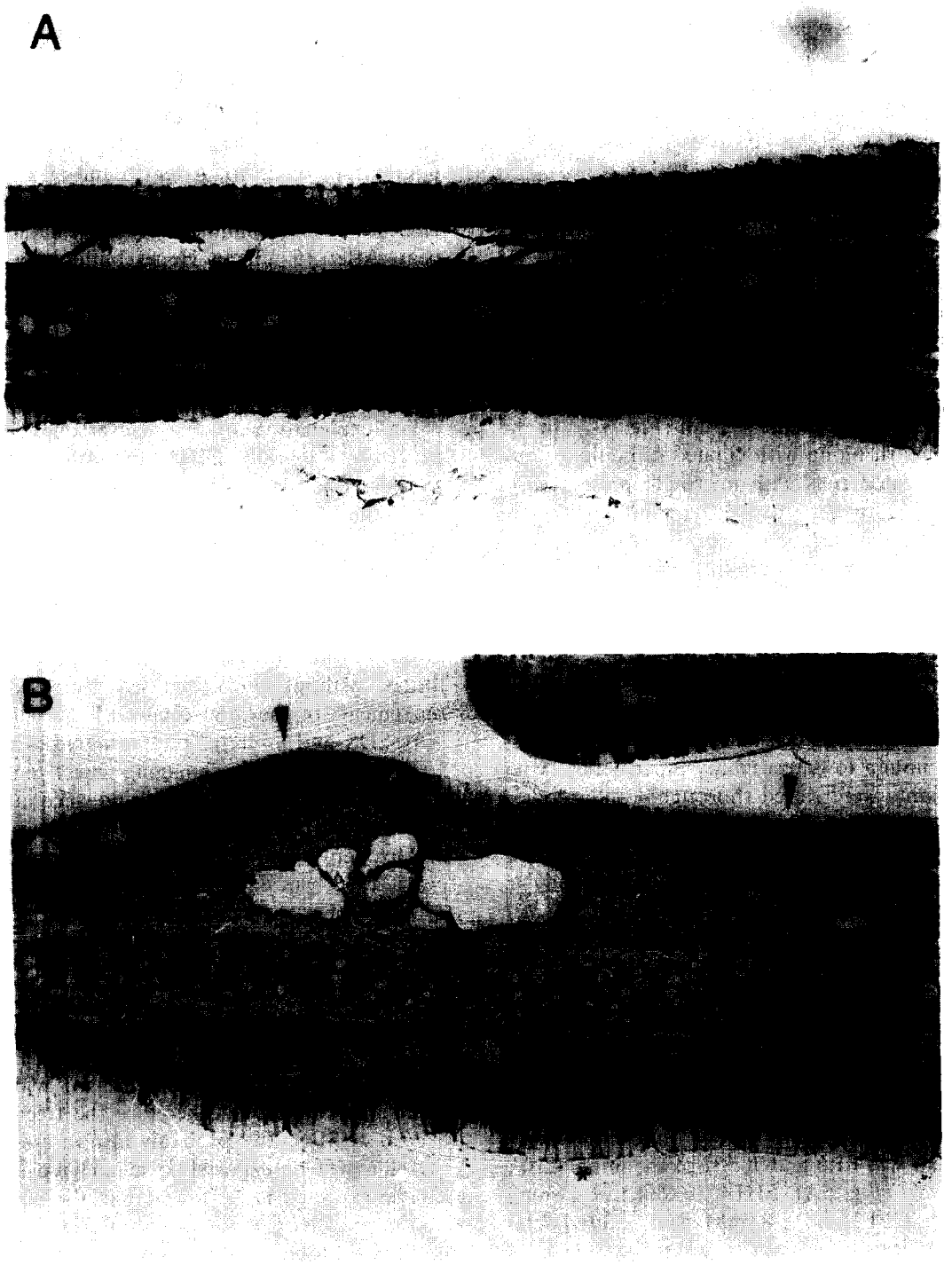

Fig. 2. Microphotograph of cross-sectioned nasal septal wall from the anterior part of the nasal cavity. (A) Columnar epithelium with cilia and goblet cells in an unreated control rat with no evidence of pathological findings. LM × 150. (B) Columnar epithelium with predominant goblet cells and focal squamous metaplasia in a rat treated with dextran microspheres for 4 weeks. Arrows indicate areas with focal squamous metaplasia and the asterisk indicates intact epithelium with prominent goblet cells. LM × 150.

no histopathological changes, and the changes in control animals administered with physiological saline and water were minor. No findings related to the substances administered were observed in the middle and posterior parts of the nasal cavity. Prominent lymphoid tissue was observed among all animals except for the two untreated control rats.

3.2. Scanning electron microscopic examination

Examination in SEM revealed no differences between the controls and the delivery systems, dextran microspheres and EHEC. In all specimens, both at 2 and 4 weeks exposure, intact ciliated cells could be seen from the anterior to the posterior part of the septum (Fig. 3A). In the anterior part, along with ciliated cells, there were also unciliated cells with microvilli present (Fig. 3B). What appears to be lymphatic cells are present on the surface of septum in the anterior part in all groups, even the untreated control group (Fig. 4).

3.3. Ciliary beat frequency measurements

When the gelling EHEC system was applied a gel formed immediately, but the gel was not transparent enough to detect ciliary movements. After the explants were rinsed thoroughly and allowed to recover in Hepes buffer for 15 min no ciliary movement could be detected. Instead, the ingredients in the gel system were tested individually in Hepes buffer. Insulin is added to the polymer solution as the commercially available Actrapid®. Therefore there are also small amounts of glycerol and *m*-cresol in this system. Neither insulin, 30 IU/ml, nor glycerol, 4.8 mg/ml, had an effect on the CBF after 15 min exposure. The preservative *m*-cresol, 0.9 mg/ml, and Actrapid® diluted as in the gel system, caused ciliostasis after 15 min. After 15 min recovery in Hepes buffer, the ciliary movement was 81.6 and 70.8% of the initial CBF, respectively (Fig. 5A). EHEC (0.6%) had a small effect on CBF, and 1.8 mM SDS caused irreversible ciliostasis. However, when the SDS concentration was decreased to 0.9 mM the CBF was not affected (Fig. 5B).

Dextran microspheres were powdered onto the explant that had been depleted of its bathing solution. An excess of spheres was added to ensure complete drainage of fluid from the tracheal ring. During exposure it was not possible to measure CBF due to the opaque gel formed when the 10 mg of microspheres absorbed the remaining liquid, approx. 25 μl. After 15 and 30 min exposure, the preparation was thoroughly rinsed with Hepes buffer and immediately the cilia started to beat. After 15 min in Hepes buffer, the beating frequency was equal to the initial CBF (Fig. 6).

4. Discussion

An explanation of the irreversible ciliostasis caused by the gelling EHEC solution might be the hypoosmotic nature of this system. The osmolarity of the EHEC system is close to the osmolarity of pure water and exposure to water caused swelling and disruption of the tracheal cells within 15 min. van de Donk et al., 1980b showed that the viability of explants depends on salts and glucose in an isotonic solution. Hypoosmotic solutions without glucose caused decreased ciliary movement. In the individual CBF measurements only SDS and *m*-cresol of the ingredients in the EHEC system had damaging effect on ciliary movements. This is not surprising since numerous studies show ciliotoxic effects of preservatives and surface active agents (Batts et al., 1990; Hermens et al., 1990; van de Donk et al., 1981). The effect of *m*-cresol is reversible but the effect of SDS is irreversible. However, when these agents are present in the gelling system they participate in the formation of the gel matrix (Pereswetoff-Morath and Edman, 1995b). It has been shown that the release of SDS from the gelling EHEC is quite slow, approx. 25% is released within 1 h (Lindell et al., 1991). Therefore the free concentration of SDS, and probably also *m*-cresol, that the cilia will be exposed to in an vivo situation can be expected to be lower than the concentration in the EHEC system. It should be noted that an SDS solution of 50% lower concentration than in the gelling system does not inhibit the ciliary movements (Fig. 5b). It can therefore be con-

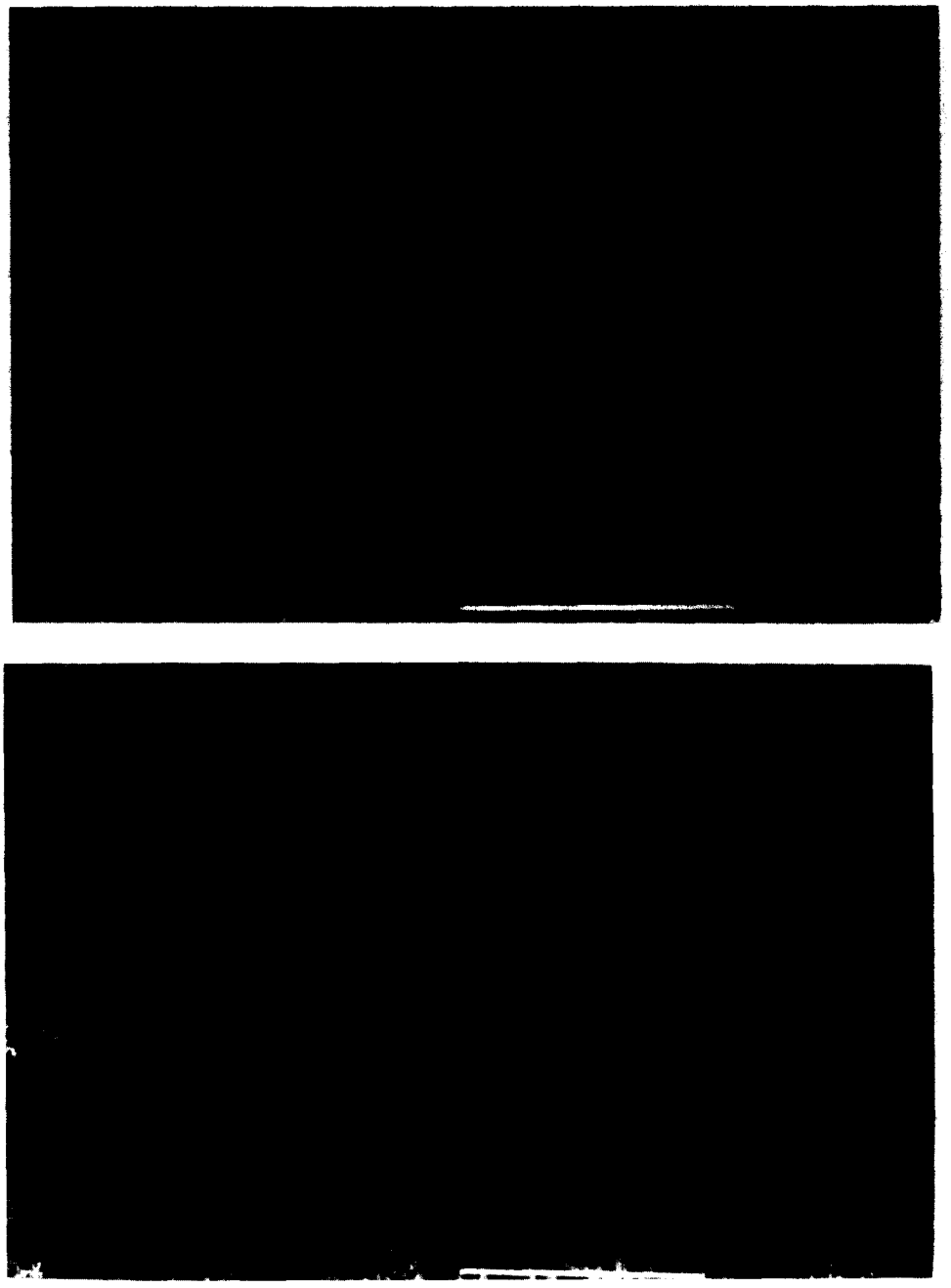

Fig. 3. Microphotographs of the septal wall in the scanning electron microscope from animals treated with 1% EHEC solution contalning 3 mM SDS. (A) Intact ciliated cells at the anterior part of the septum after 4 weeks of treatment. (B) Intact ciliated cells and non-ciliated cells with microvilli at the septum, close to the vestibule after 2 weeks of treatment. The bars denote 10 μm.

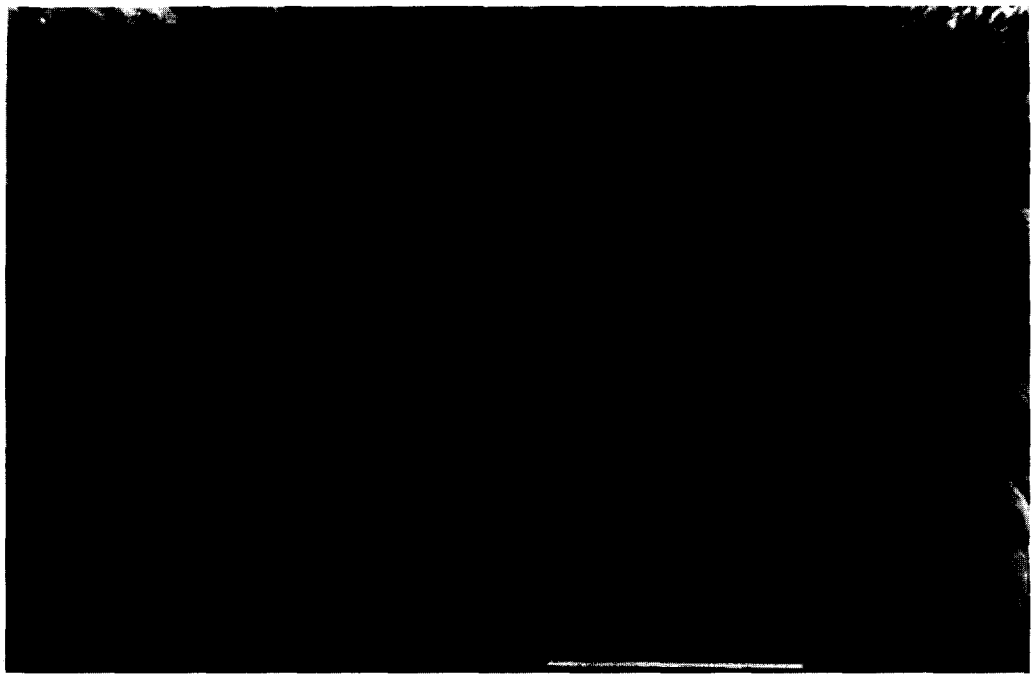

Fig. 4. Microphotograph by scanning electron microscope of the septal wall in one animal treated with 1.8 mM SDS in physiological saline for 4 weeks. Lymphatic cells on the surface of the ciliated cells at the anterior part of the septal wall. The bar denotes 10 μm.

cluded that the ciliotoxic effect of the EHEC system detected in vitro, largely depends on the hypoosmotic nature of the solution.

The dehydration by dry microspheres of the surroundings of the explant in the CBF measurements was expected to cause ciliostasis, and perhaps damage to the cilia, due to depletion of ions and glucose. The immediate recovery of the ciliary movement indicates that the cilia are not damaged by the dextran microspheres. The reason might be that the periciliary fluid is held close to the cell surface and is therefore not absorbed by the spheres, or that the formed gel layer of dextran spheres maintains an environment that is humid enough to keep the cilia intact.

Slight to moderate hyperplasia of the respiratory epithelium at the septal wall in the anterior part of the nasal cavity could be seen after 4 weeks of administration of dextran microspheres. This effect could not be detected in the middle and posterior parts of the cavity. The respiratory hyperplasia at the septal wall in the present study resembles the findings in rabbits administered with 10 or 20 mg degradable starch microspheres (DSM) intranasally for 4 or 8 weeks (Bjork et al., 1991). A plausible explanation of this effect could be that the concentration of spheres is high in this area immediately after administration, resulting in compensatory hyperplasia. The hyperplasia, especially the goblet cell hyperplasia, may also be a response to local dehydration, since water is absorbed from the mucosa to the spheres. Controls that received 1.8 mM SDS in physiological saline for 4 weeks showed hyperplasia of the respiratory epithelium comparable to the findings in rats administered with dextran microspheres. However, rats subject to administration of 0.6% EHEC with 1.8 mM SDS and Actrapid® showed no histopathological changes despite the same SDS concentration as the plain solution. The thermogelling EHEC system appears to protect the cells from local effects of SDS exposure. This is in agreement with the results from the in vitro ciliotoxicity test, i.e., the actual concentration the epithelial cells are exposed to is lower than 1.8 mM. The mild effects of the 1% EHEC system

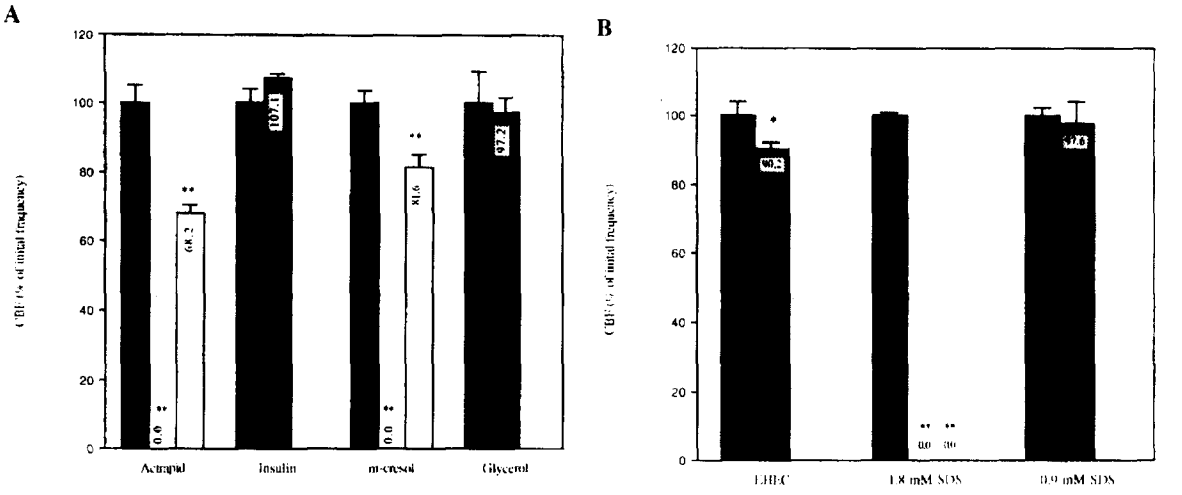

Fig. 5. The effect of the ingredients in the gel system on ciliary beat frequency (CBF) in vitro. Black stipples, inital CBF; grey stipples, CBF after 15 min exposure; and white stipples, CBF after 15 min recovery. (A) Actrapid® and its ingredients individually. (B) The ingredients of the polymer system, i.e. EHEC and SDS individually. $^{**}P < 0.01$, $P < 0.05$, Student's t-test.

may be caused by the higher concentration of SDS in this system, which leads to a higher free concentration of SDS in the nasal cavity.

Prominent lymphoid tissue of, in general, similar degree of severity was seen among all groups except for the undosed control group. The prominence of the lymphoid tissue may be an expression of lymphoid hyperplasia. As all animals, except for the undosed controls, have been anaesthetised, the prominence of the lymphoid tissue might be a local response to halothane exposure. This has not been reported previously and halothane is considered as a safe inhalation anaesthetic for humans. However, it has been reported that both humans and rats subject to repeated halothane exposure showed pathological alterations of liver tissue (Keeley et al., 1970; Ross Jr. and Cardell Jr., 1972). In a review by Chang and Katz (1976) the authors conclude that, since the pathological changes in tissue involve the cytomembrane system, i.e. plasma membrane, mitochondria, nuclear membrane, endoplasmatic reticulum and Golgi complex, it is not unreasonable to expect that halothane would affect biological membranes.

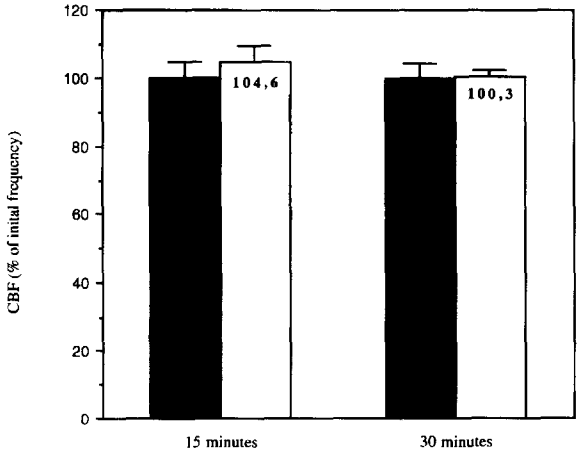

Fig. 6. The effect of dextran microspheres on ciliary beat frequency (CBF) in vitro after 15 and 30 min exposure, respectively, and thereafter 15 min recovery. Black stipples, inital CBF; and white stipples, CBF after first 15 or 30 min exposure and thereafter 15 min recovery.

In conclusion, the effects on nasal mucosa seen after repeated administration of dextran microspheres and thermogelling EHEC are located in the anterior part of the nasal cavity. The reason might be that the delivery systems are deposited there, and therefore this area is exposed to dehydration by the spheres and SDS exposure by the EHEC system. However, the observed effects were modest, so both dextran microspheres and thermogelling EHEC can be considered as relatively safe vehicles for nasal administration of drugs.

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