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Nasal Absorption of Desmopressin in Rats and Sheep. Effect of a Bioadhesive Microsphere Delivery System

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Abstract—The nasal absorption of desmopressin was studied in two animal models, the rat and the sheep. The bioavailability after nasal administration was found to be 13 times higher in the rat model. This discrepancy is suggested to be due to the impaired mucociliary clearance mechanism in the rat model and possibly differences in enzymatic degradation and elimination rates of the drug. The effect of the addition of L-α-lysophosphatidylcholine (LPC) to the formulations as an absorption enhancer was most pronounced in the sheep model. The use of the bioadhesive starch microsphere delivery system, especially in combination with LPC, had a profound effect on the absorption of desmopressin in sheep, with bioavailabilities reaching nearly 10% compared with 1·2% for a simple nasal solution of desmopressin.

Desmopressin, a synthetic derivative of the posterior pituitary hormone arginine vasopressin, is widely used clinically in the treatment of central diabetes insipidus. Desmopressin has traditionally been administered by injection but in recent years formulations for the nasal and oral routes of administration have been marketed. However, compared with parenteral administration, the bioavailabilities for these two routes of administration are generally low. For oral delivery, bioavailabilities in the order of 0·1% have been found in studies in healthy adults after administration of a 200-μg dose of desmopressin (Fjellestad-Paulsen et al 1993). Variable absorption of desmopressin has been reported after nasal administration of a 20-μg dose with bioavailabilities ranging from as low as 2·0% (Kohler & Harris 1988) and 3·4% (Fjellestad-Paulsen et al 1993) to 11·3% (Vilhardt & Lundin 1986) in healthy and in hydrated volunteers (the last study), respectively. Other studies have shown that the bioavailability obtained after nasal administration is highly dependent on the concentration and dose volume of the desmopressin formulation and the delivery device used (Harris et al 1986, 1988). The relatively low absorption of desmopressin across mucosal surfaces is most likely due to the hydrophilic nature of the peptide, its relatively large size and degradation by peptidases in the mucosa. To improve the bioavailability, various groups have explored the feasibility of employing absorption enhancers and enzymatic inhibitors for the improvement of the transport of desmopressin across the nasal membrane (Olanoff et al 1987; Morimoto et al 1991a, b).

Recently, the concept of using bioadhesive delivery systems in the form of degradable starch microspheres, hyaluronic ester microspheres and chitosan for improvement of nasal absorption of peptides and proteins was introduced by Illum and coworkers (Illum et al 1987, 1988, 1989, 1990, 1994; Farraj et al 1990). The bioavailability of insulin in sheep was found to be improved more than tenfold when administered in combination with a starch microsphere formulation. This enhancement was improved even further to thirtyfold when the lysolecithin, L-α-lysophosphatidylcholine (LPC) was introduced into the freeze-dried microsphere formulation (Farraj et al 1990). It has been shown by various authors that the nasal starch microsphere system has no adverse effect on the nasal membrane and that it achieves its effect by a combination of bioadhesion and a transient opening of the tight junctions (Edman et al 1992). LPC is a surface-active, amphiphilic compound present in biological membranes. It has recently been investigated as an enhancer for the intranasal absorption of insulin in rats (Illum et al 1988, 1989, 1990; O'Hagan & Illum 1990; Fisher et al 1991). The advantages of using LPC compared with other enhancer systems have been discussed in these publications.

The aim of the present work was to establish the absorption characteristics of desmopressin following nasal administration in two animal models, the rat and the sheep. Further studies were performed to evaluate the effectiveness of the starch microsphere system and LPC either alone or in combination, for the improvement of the nasal absorption of desmopressin.

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Materials and Methods

Materials

1-Deamino-8-D-arginine vasopressin (desmopressin) was obtained as a gift from Ferring AB, Malmo, Sweden, together with iodinated desmopressin and antiserum (ADA-6) for the radioimmunoassay (RIA). Degradable starch microspheres manufactured by emulsion polymerization of hydrolysed potato starch was obtained from Kabi Pharmacia AB (Uppsala, Sweden). L-α-Lysophosphatidylcholine (LPC) was obtained from Sigma, Poole, UK. All other materials were of reagent grade and obtained from Merck, Sweden or Sigma, UK. Water for preparation of RIA reagents was from a Millipore purification system.

Preparation of desmopressin formulations

For the studies in rats, desmopressin was dissolved in phosphate-buffered saline (PBS) (pH 7·4) at a concentra-

tion of 20 or 50 $\mu g\,mL^{-1}$ for intranasal formulations and of 2 or 5 $\mu g\,mL^{-1}$ for intravenous formulations. For the studies in sheep, nasal formulations of desmopressin (300 $\mu g\,mL^{-1}$) were prepared in PBS (pH 7·4). When required, the enhancer LPC was added to the PBS at a concentration of 2 $\mu g\,mL^{-1}$. The solutions were prepared on the day of the experiments. Microspheres containing 2 μg desmopressin mg^{-1} starch microspheres were prepared by dissolving the appropriate amount of drug in PBS, mixing with the starch microspheres and incubating for 1 h at room temperature before freeze-drying for 48 h on a Modulyo 4 K freeze-drier to produce a light, fluffy, free-flowing powder. Microsphere formulations containing LPC were prepared by addition of 75 μg LPC mg^{-1} microspheres in the microsphere suspension before freeze drying.

Rat studies

Anaesthetized male Wistar rats, 200 g (Animal Unit, Nottingham University, UK), were used in all experiments. Anaesthesia was induced by an intraperitoneal injection of sodium pentobarbitone (Sagatal) 60 mg kg^{-1} and maintained by additional doses of 15 mg kg^{-1} as required. The surgical procedure included the performance of tracheostomy to maintain respiration during anaesthesia, cannulation of the carotid artery for collection of blood samples and cannulation of the jugular vein for fluid replacement or intravenous administration of desmopressin drug formulation as described by Chandler et al (1991). Rats in groups of four received either 5 or 12·5 $\mu g\,kg^{-1}$ desmopressin in a volume of 50 μL in one nostril using a polypropylene tubing attached to a 100-μL Hamilton microsyringe. For intravenous administration, the rats received 0·5 or 1·25 $\mu g\,kg^{-1}$ desmopressin given as a bolus injection into the jugular vein. In addition PBS was administered as a control to ensure no interference with the RIA or the desmopressin. Blood samples (400 μL) were withdrawn from the carotid artery into Eppendorff tubes containing 10 μL heparinized saline (150 int. units mL^{-1}) at 0, 5, 15, 30, 45, 60, 120, 180, 240 and 300 min after nasal administration and at 0, 0·5, 3, 5, 10, 30, 45, 60, 120, 180, 240 and 300 min after intravenous dosing. The samples were kept on ice and plasma separated within 10 min of sampling. The plasma samples were then kept at −20°C until analysis.

Sheep studies

Female cross-bred Suffolk & Texel sheep, 35–45 kg, with free access to food and water, were divided into groups of three. Before the experiments, one of the external jugular veins was cannulated in each sheep with an indwelling Viggo secalon catheter for blood sampling and intravenous administration. The catheter was kept patent by flushing with heparinized saline and removed upon completion of the study. For intranasal administration the sheep were sedated by an intravenous dose of ketamine hydrochloride (2 mg kg^{-1}) to prevent sneezing during administration. The sedation lasted for about 3 min. The methods of intranasal administration of liquid and powder formulations to sheep have been described previously (Illum et al 1988). The aqueous desmopressin formulations were administered in a volume of 500 μL with and without LPC (0·2% w/v). The lyophilized formulations were similarly administered at 5 μg desmopressin kg^{-1} with a dose of microspheres of 2·5 mg kg^{-1}. For intravenous studies the sheep received 0·5 $\mu g\,kg^{-1}$ given as a bolus injection into the jugular catheter. After dosing, blood samples were collected from the jugular vein into heparinized tubes kept on ice at 0, 5, 15, 20, 30, 40, 50, 60, 90, 120, 150, 180, 240 and 300 min after administration for intranasal administration and at 0, 1, 3, 5, 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 240 and 300 min after administration for intravenous injection. Plasma was separated by centrifugation (3000 rev min^{-1}, 4°C, 5 min) and samples stored frozen at −20°C until extraction and analysis.

Radioimmunoassay (RIA) of desmopressin

Before analysis of the blood samples for content of desmopressin, it was necessary to extract the drug using a method employing the precipitation of protein with acetone and the removal of lipids with petroleum ether. Two hundred microlitres of the blood sample or control and 300 μL 0·6 M sodium chloride were added to polypropylene tubes kept on ice. One millilitre ice-cold acetone was added and the samples vortexed and centrifuged (3000 rev min^{-1}, 4°C, 15 min). The supernatant was decanted into a new polypropylene tube and extracted twice with 2 mL ice-cold petroleum ether. The samples were centrifuged (3000 rev min^{-1}, 4°C, 10 min) and the upper organic phase removed. The remaining aqueous phase was evaporated to dryness in a Savant Speed Vac Concentrator. The efficiency and reproducibility of the procedure was determined by control extraction of plasma containing 100 or 500 pg mL^{-1} desmopressin. For RIA, samples were reconstituted in 1 mL assay buffer. The assay buffer was prepared as a 0·1 M sodium phosphate buffer (pH 7·5) containing 0·1% human serum albumin (HSA), 0·02% sodium azide and 0·01% Triton-X 100. Serum-coated charcoal was prepared by mixing 5 g L^{-1} charcoal with 25 mL L^{-1} human serum in assay buffer (without Triton-X) overnight at 4°C.

For the RIA, 200 μL test sample, standard solution or assay control, 100 μL [^{125}I]-desmopressin and 100 μL antisera ADA-6 (1/75000) were added to polystyrene assay tubes in triplicate, vortexing after each addition and incubated at 4°C for 48 h. Total activity tubes (T) containing 100 μL [^{125}I]-desmopressin and 800 μL assay buffer, non-specific binding tubes (NSB) containing 300 μL assay buffer and 100 μL [^{125}I]-desmopressin and maximum binding tubes (B_0) containing 200 μL assay buffer and 100 μL [^{125}I]-desmopressin were also prepared and incubated as above. After incubation, 500 μL charcoal/serum suspension was added to all tubes except T. The tubes were then vortexed and centrifuged (3000 rev min^{-1}, 4°C, 15 min), to separate bound and free radioactivity. The resultant supernatant was decanted into empty assay tubes and the activity counted in a gamma counter (LKB, Clinigamma 1272 Twin Detector). Standard curves were produced using a standard sample of desmopressin in concentrations from 2·5 to 1280 pg mL^{-1}. The maximum % binding (% B_{max}) was calculated by comparing the activity in B_0 samples with the total activity tubes as defined by:

$$\frac{B_0 - NSB}{T} = \%\ B_{max} \qquad (1)$$

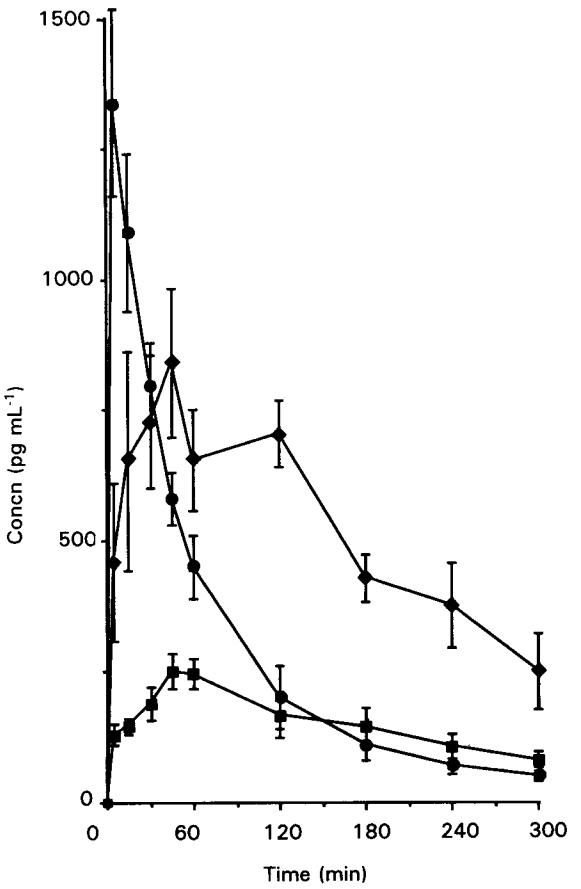

FIG. 1. Nasal administration to rats of desmopressin in simple solutions (■ 5 and ◆ 12·5 μg kg^{-1}) and in solution (5 μg kg^{-1}) combined with 0·2% LPC (●). All values are mean ± s.e.m.

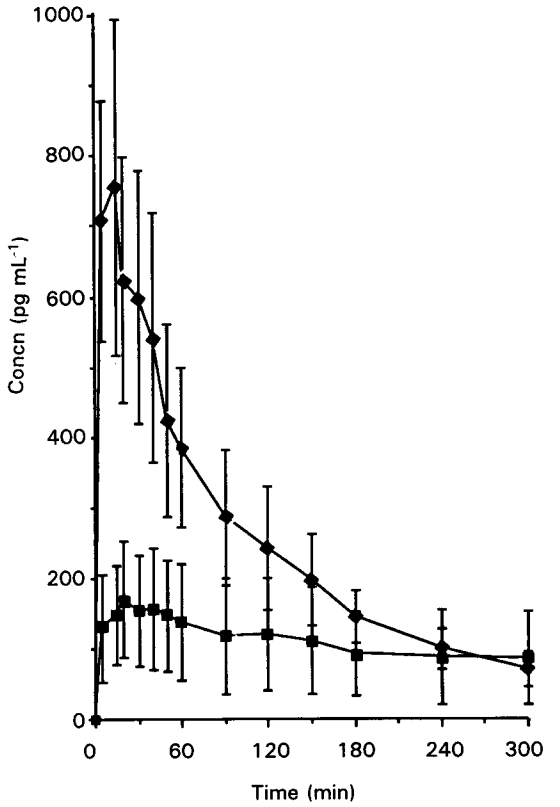

FIG. 2. Nasal administration to sheep of desmopressin in simple solutions (■ 5 μg kg^{-1}) and a solution (5 μg kg^{-1}) combined with 0·2% LPC (◆). All values are mean ± s.e.m.

Binding of 50% and above was considered to represent satisfactory performance of the assay.

Results

The mean plasma concentrations of desmopressin vs time following nasal administration to rats at two dose levels (5 and 12·5 μg kg^{-1}) are shown in Fig. 1. Plasma levels rose rapidly over the first 15 min of sampling with a C_{max} of 0·27 and 0·91 ng mL^{-1} for the low and the high doses, respectively, with t_{max} at between 30 and 45 min. Thereafter, drug plasma levels gradually declined. A dose-dependent increase in both the mean C_{max} and AUC_{0-300} values was observed. Selected pharmacokinetic parameters are given in Table 1.

The bioavailabilities were found to be 15·8 and 19·5% for the 5 and the 12·5 μg kg^{-1} doses, respectively. The addition of the absorption enhancer, LPC, in a concentration of 0·2% w/v improved the absorption of desmopressin with a fivefold increase in C_{max} and nearly a doubling in bioavailability. When administered intranasally to sheep as a simple solution, desmopressin was only poorly absorbed with a bioavailability of 1·2% (Fig. 2, Table 2). The addition of LPC to the formulation at a concentration of 0·2% w/v resulted in a significant improvement with a bioavailability of 3·2% compared with intravenous injection. Plasma levels rose to a C_{max} of 0·80 ng mL^{-1} within 12 min.

The use of starch microspheres as a nasal powder delivery system in sheep resulted in an increase in C_{max} from 0·17 to 1·74 ng mL^{-1}, an increase in AUC from 44·6 to 172·2 ng mL^{-1} min and a bioavailability of 4·7%. The

Table 1. Nasal administration of desmopressin in rats. Pharmacokinetic parameters.

Formulation	Route	Dose (μg kg^{-1})	MRT (min)	C_{max} (ng mL^{-1})	t_{max} (min)	AUC_{0-300} (ng min mL^{-1})	F (%)
Solution	Nasal	5	157·3 ± 11·4	0·27 ± 0·05	45·0 ± 12·3	54·4 ± 7·9	15·8 ± 4·6
		12·5	144·8 ± 6·6	0·91 ± 0·26	30·0 ± 15·0	167·5 ± 21·3	19·5 ± 4·3
Solution with LPC	Nasal	5	84·4 ± 10·1*	1·41 ± 0·18*	7·5 ± 2·9*	89·2 ± 23·2*	25·9 ± 6·7*
Solution	Intravenous	0·5	97·2 ± 15·9	—	—	35·9 ± 9·4	100·0 ± 0·0
		1·25	67·1 ± 11·6	—	—	86·1 ± 11·8	100·0 ± 0·0

*$P < 0·05$ compared with control solution.

Table 2. Nasal administration of desmopressin to sheep. Pharmacokinetic parameters.

Formulation	Route	Dose ($\mu g\,kg^{-1}$)	MRT (min)	C_{max} ($ng\,mL^{-1}$)	t_{max} (min)	AUC_{0-300} ($ng\,min\,mL^{-1}$)	F (%)
Solution	Nasal	5	190·3 ± 35·9	0·17 ± 0·08	25·0 ± 7·6	44·6 ± 53·1	1·2 ± 1·4
LPC	Nasal	5	178·4 ± 81·9	0·80 ± 0·19	11·7 ± 3·3*	117·4 ± 77·1	3·2 ± 2·1
Starch microspheres	Nasal	5	131·8 ± 1·8*	1·74 ± 0·16*	8·3 ± 3·3*	172·2 ± 17·3*	4·7 ± 0·5*
Starch microspheres with LPC	Nasal	5	133·2 ± 17·2	2·74 ± 0·15*	18·3 ± 1·7	352·5 ± 102·2*	9·6 ± 2·8*
Solution	Intravenous	0·5	121·3 ± 5·8	—	—	368·9 ± 42·3	100·0 ± 0·0

*$P < 0·05$ compared with control solutions.

maximum plasma levels were reached rapidly with a t_{max} of 8·3 min (Fig. 3). The plasma levels remained elevated for 5 h. The combination of starch microspheres and LPC proved to be a potent nasal delivery system for desmopressin with an increase in C_{max} to 2·74 ng mL^{-1}, an AUC of 352·5 ng mL^{-1} min and a bioavailability of 9·6%. For this system the obtained peak was broader and plasma levels did not reach basal levels within the 5-h sampling period.

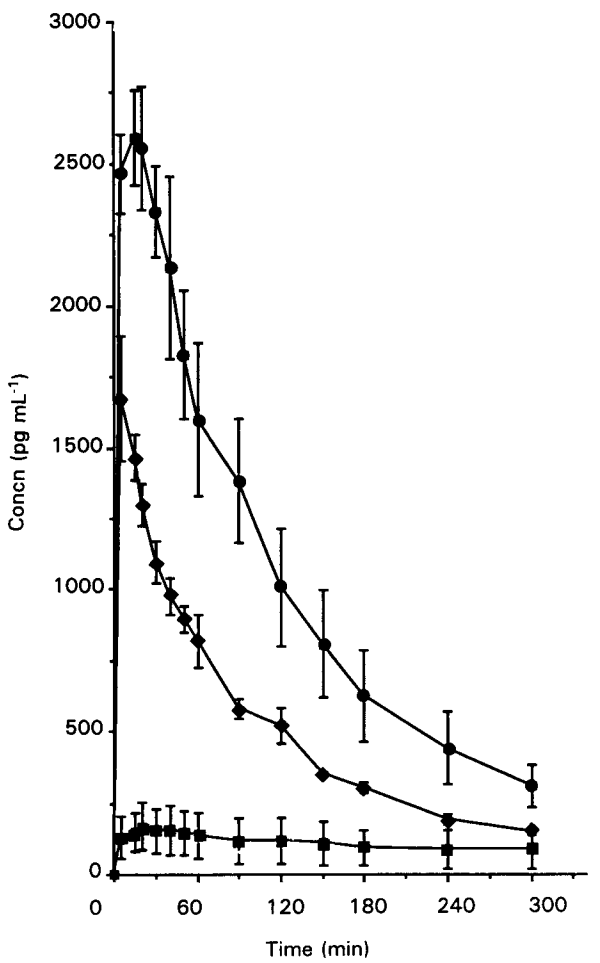

FIG. 3. Nasal administration to sheep of desmopressin (5 $\mu g\,kg^{-1}$) in a simple solution (■), as a freeze-dried powder with starch microspheres (◆) and as a freeze-dried powder with starch microspheres and LPC (●). All values are mean ± s.e.m.

Discussion

Desmopressin as a simple solution was shown to be rapidly absorbed from the nasal mucosa of rats with relatively high bioavailabilities (15·8–19·5%) obtained for the two dose levels. The dose-dependent AUC values indicate that the absorption across the nasal membrane is a first-order process. This is supported by results in man where deconvolution analysis similarly demonstrated first-order absorption of the peptide (Harris et al 1988). However, it should be noted that individual plasma profiles showed marked variation amongst animals as has previously been observed in human studies (Andersson et al 1988). In the sheep, the nasal absorption of the desmopressin solution was low, with a bioavailability of 1·2%, demonstrating a considerable species difference. Similar differences have been shown for nasal absorption of simple solutions of human growth hormone (hGH) in anaesthetized rats, in conscious rabbits and in sheep, with bioavailabilities of 2·3, 1·4 and 0·2%, respectively (Fisher et al 1991). However, it should be noted that in the study of Fisher et al (1991) the doses administered nasally to the three animal models varied from 0·9 int. units kg^{-1} in the rabbit and sheep to 2·93 int. units kg^{-1} in the rat. It has been suggested that the most likely explanation for this species variation is the impaired mucociliary clearance mechanism in the anaesthetized rat model compared with the conscious sheep model (Fisher et al 1991). Furthermore, absorption could also be influenced by differences in the proteolytic enzymes present in the nasal lumen and mucosa and in the elimination rates of desmopressin in the animal models. The addition of LPC to the desmopressin solutions resulted in about a fivefold increase in C_{max} after nasal absorption in both animal species. However, the effect of LPC on the bioavailability was more pronounced in the sheep model (2·6 times) than in the rat (1·6 times), although the overall bioavailability in sheep remained significantly lower. A similar pattern was found by Fisher et al (1991) for the improvement of nasal hGH absorption in rats and sheep with LPC, the highest effect seen in the sheep model. Baldwin et al (1990) found that the bioavailability of hGH in sheep increased by a factor of two compared with the rat, when the enhancer sodium taurodihydrofusidate was added to the nasal formulations.

The lower bioavailability for desmopressin found in sheep compared with rat, even in the presence of LPC, supports the view that mucociliary clearance is an important mechanism for reducing the absorption of nasally administered

drugs. However, the variable efficiency of LPC in the two animal models, the enhancing effect being highest in the sheep model, could also reflect differences in ability for the enhancer to interact with the mucosal membranes of the two species. The haemolytic effects of LPC on erythrocytes from various animal species have been shown to correlate with the lipid composition of the erythrocyte membrane (Weltzien 1979; Ishii et al 1989). However, since sheep erythrocytes, which consist almost exclusively of sphingomyelins, were shown to be only a little susceptible to damage whilst rat erythrocytes were readily haemolysed, the membrane interaction is probably not the major determining factor for the enhancing effect of LPC in the two animal species.

The use of bioadhesive starch microspheres as a nasal delivery system for desmopressin significantly improved the absorption of drug, both in terms of peak plasma levels and bioavailability. Similar effects have previously been found in sheep for insulin, with a 4·5-fold increase in peak levels compared with administration of a simple nasal insulin solution (Farraj et al 1990), as well as for hGH (Illum et al 1990) and gentamicin (Illum et al 1988). The microsphere delivery system has been suggested to exert its effect by two mechanisms (Illum et al 1994). Firstly, due to the bioadhesive properties of the starch microspheres, the rate of clearance of the formulation will be decreased, thereby allowing a longer time of contact between the drug and the absorptive sites in the nasal membrane. Secondly, in a study employing monolayers of Caco-2 cells for the transport of mannitol and insulin, it was shown that microspheres applied to the cell surface promoted a transient widening of the tight junctions between the cells (Edman et al 1992). The effect was suggested to be due to the swelling of the dry microsphere powder when in contact with the membrane, resulting in a dehydration and shrinkage of the cells, or possibly an increased water flux through the tight junctions. A similar effect could be envisaged to occur in the nasal membrane after applying the freeze-dried microsphere delivery system by promotion of the paracellular transport of desmopressin. This latter mechanism is supported by the pulsatile shape of the absorption profile, which is not dissimilar to the profile obtained after intravenous injection of desmopressin.

The combination of starch microspheres and LPC showed a significant improvement in bioavailability (9·6%) in comparison with the microsphere delivery system (two-fold) and the LPC solution formulation (threefold) when administered singly. Similar effects have been found for insulin, where the bioavailability obtained with a similar combined system was 13·1% (Farraj et al 1990). These results show that a synergistic effect exists between the effect of the microspheres and the effect of the LPC, i.e. the effect of the LPC being further improved by the microsphere system.

LPC can be considered to be a mild enhancer that exhibits detergent-like properties (Weltzien 1979). Its absorption promoting activity may be related to its ability to disrupt lipid-protein interactions within the membrane and subsequent structural re-organization of the bilayer. This could occur as a result of solubilization of membrane lipids or be due to increased membrane fluidity following incorporation of LPC monomers into the bilayer as seen for bile salts and acylcarnitines (Gibaldi & Feldman 1970; Inoue & Kitagawa 1974; Weltzien 1979; LeCluyse et al 1991). Recently, didecanoyl-L-phosphatidylcholine has been shown to alter the tight junction integrity when applied in-vitro to an excised rabbit nasal membrane mounted in an Ussing chamber (Vermehren et al 1993). A similar effect on the membrane would be expected for LPC. In addition, in the gut LPC has been found to decrease the viscosity and elasticity of the mucus layer and alter transmucosal water flux (Martin et al 1978; Ammon et al 1983) and similar effects could be expected to reduce the barrier function of the nasal mucus. The histological effects of LPC solutions on the epithelial cells after contact with rat nasal mucosa for 60 min were shown to be similar to those of sodium taurodihydrofusidate (Chandler et al 1991). The enhancer induced mucus secretion and epithelial disruption in terms of reduction in cell layer thickness and some intracellular rearrangement. The epithelial cell layer was not severely damaged as has been shown for non-ionic surfactant enhancers such as laureth-9.

It can be concluded that the nasal microsphere delivery system was able to promote significantly the absorption of desmopressin after nasal application and that this effect could be further improved by the addition of a mild enhancer system in the form of LPC. A similar improvement of the absorption after administration in man would be highly beneficial.

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