Nasal Absorption in the Rat. III. Effect of Lysophospholipids on Insulin Absorption and Nasal Histology

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The intranasal absorption enhancing and histological effects of a range of lysophospholipids has been investigated in the rat. Blood glucose levels fell rapidly following the administration of insulin (8 IU/kg) in combination with lysophosphatidylcholines (LPC; 0.625% w/v) which had ten or more carbon groups in their fatty acid chain. The effect of the LPC-caproyl (C6) was comparable to that of an unenhanced insulin formulation; the enhancing effect of LPC-decanoyl (C10) was similar to that of an LPC-palmitoyl/stearoyl (C16/C18) for similar concentrations. The effect of LPC-decanoyl was reduced with concentration but was still significant at 0.2% w/v (5mM). Lysophosphatidylglycerol (LPG) had a marked insulin absorption enhancing effect even at 0.0625% w/v. The histological effects of LPC-caproyl were similar to those of an unenhanced insulin formulation, while co-administration of LPC-decanoyl resulted in evidence of epithelial interaction. LPG (0.5% w/v) resulted in similar histological changes as LPC (0.625% w/v) (1), but at 0.0625% w/v no significant changes in epithelial integrity were observed. The length of the fatty acid residue of lysophospholipids was identified as an important factor for intranasal absorption enhancing activity. The nature of the polar head group may also have an influence. Increased insulin absorption was not necessarily accompanied by severe disruption of the nasal epithelium. Careful selection of lysophospholipid type and concentration may enable therapeutic drug levels to be achieved via the nasal route without prohibitive toxic effects.

KEY WORDS: intranasal administration; insulin; absorption enhancer; nasal histology; lysophospholipids.

INTRODUCTION

A variety of different agents have in recent years been investigated as nasal absorption enhancers for administration of otherwise poorly absorbed peptide and protein drugs (2-9). Unfortunately, the achievement of increased plasma drug levels have often also been associated with membrane irritation or damage. Effects such as removal of membrane proteins, epithelial cell loss, excessive mucus discharge, ciliotoxicity and the possible disturbance of the normal enzymatic balance of the mucosa have to be considered in the selection of nasal absorption enhancers.

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These toxicity concerns prompted the development of a method which enabled the controlled assessment of the histological effects of different enhancing agents. In this method treated nasal tissue was compared with untreated nasal tissue in the same animal employing the two sides of the nasal cavity separated by the nasal septum (10). In a later study the nasal toxicity model was combined with absorption experiments using insulin as a model polypeptide drug (1). This simultaneous assessment of absorption enhancement and histological effects enabled the relationship between enhancing efficacy and mucosal disruption to be investigated. In this study correlation between enhancing efficiency and epithelium disruption was found for the non-ionic surfactant laureth-9. However, the removal of the epithelial barrier was not considered to be the only mechanism of action for all of the enhancers investigated. For instance, lysophosphatidylcholine (LPC), the natural surfactant, was found to result in good insulin absorption enhancement with only moderate disruption of the nasal epithelium in the rat model although in *in vitro* tests LPC has been found to be ciliotoxic (1, 11) after applying ciliated tissue to a 0.5% LPC solution. This latter toxicity test can be considered very aggressive.

The LPC preparation was derived from egg-lecithin and contained a mixture of side chain lengths, primarily palmitoyl (16 carbons) (72%) and stearoyl (18 carbons) (24%) (12). Individually, both LPC-stearoyl and LPC-palmitoyl have been shown to result in the reduction of blood glucose concentrations to a similar extent as the mixed preparation following intranasal administration with insulin (16.7 IU/kg) at a concentration of 0.5% w/v (12). LPG is closely related in structure to LPC, differing only in the nature of the polar head group (Figure 1). At a concentration of 0.5% w/v, LPG has been used as an enhancer of vaginal absorption, resulting in an increase of insulin uptake comparable to an equivalent concentration of LPC, but with fewer signs of interaction with the vaginal membranes (13). The effect of LPG on the nasal epithelium has not previously been reported.

A range of synthetic LPC homologues is available commercially in which the hydrocarbon chain attached to the glycerol backbone varies in length or degree of saturation (Figure 1). Changing the length of a hydrocarbon side chain will alter the hydrophobic nature of a compound, thus influencing its physicochemical characteristics. This in turn may affect the absorption enhancing activity as shown for bile salts by Gordon et al. (14). The effect of polyoxyethylene chain length on intranasal insulin absorption enhancement by non-ionic surfactants was described by Hirai et al. (3). Similarly, altering the fatty acid chain length of acylcarnitines influenced the rectal absorption of cefoxitin and a polypeptide (15). These examples demonstrate the importance of enhancer structure on absorption promoting efficacy.

The present studies aimed to identify the structural features of lysophospholipids important for nasal absorption enhancing activity and to clarify the significance of membrane interactions *in vivo* as a mechanism of action of these enhancers.

A range of synthetic LPC homologues to include LPC-stearoyl and LPC-caproyl with the longest (C18) and shortest (C6) carbon chain lengths respectively was chosen for

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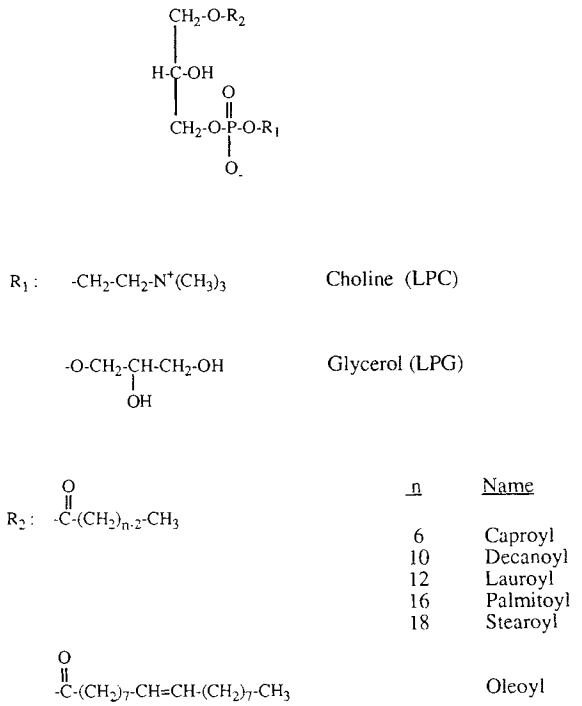

Figure 1: The structures of lysophospholipids with fatty acid chain length of 6, 10, 12, 16 and 18.

nasal absorption experiments in the rat model. As before, insulin was chosen as the peptide model drug and absorption was monitored by the effect on blood glucose levels (1). The selected LPG homologues were further investigated for possible interaction with the nasal membrane in the histological model. In addition, the effect of lysophosphatidylglycerol (LPG) was investigated for both absorption enhancing and histological activity in the nasal cavity.

MATERIALS

Semi-synthetic human insulin (sodium salt, SHI, BN P371) was obtained from Novo-Nordisk, Gentofte, Denmark; the water content was determined as 14% w/w/.

LPC-stearoyl (LPC_s; C18; mw. = 523.7), LPC-oleoyl (LPC_o; C18 unsaturated; mw. = 521.7), LPC-lauroyl (LPC_l; C12; mw. = 439.5), LPC-decanoyl (LPC_d; C10; mw. = 411.5), LPC-caproyl (LPC_c, C6; mw. 355.4) and LPG (stearoyl/palmitoyl mixture) were purchased from Sigma Chemical Company Ltd., Dorset, UK. Standard buffer salts, histological reagents and all other materials used were of reagent grade.

METHODS

Dose Preparation

Formulations were prepared for administration of the required insulin dose (8 IU/kg) in 20 µl volumes to rats of 250g body weight. Insulin solution (100 IU/ml) was freshly prepared each day in phosphate buffer (pH 7.3) and the lysophospholipid added at the required concentrations.

In the initial screening experiments for the assessment of absorption enhancing effect, LPC homologues were added for a final concentration of 0.625% w/v (0.5 mg/kg) for comparison with the previous study (1) Follow up experiments in the combined absorption and histology model used enhancers at equimolar concentrations (11.9 mM). In this model, LPG was investigated at concentrations of 0.5% w/v (0.5 mg/kg; 200g rats used) and 0.0625% w/v (0.05 mg/kg).

Animal Preparation, Dosing and Blood Sampling

Groups of four to eight animals were used for each formulation. Animal preparation and nasal dosing was carried out according to the methods described by Chandler et al. (1) for the in vivo assessment of nasal drug absorption. Male Wistar rats (JABU, Sutton Bonington, U.K.) weighing approximately 250g were fasted overnight prior to each experiment and anaesthetised intraperitoneally with 60 mg/kg sodium pentobarbitone (Sagatal, 60 mg/ml, May and Baker, Essex, U.K.) A tracheotomy diverted airflow from the nasal passages and aided breathing. The oesophagus was closed by ligation onto the tracheal cannula. The left carotid artery and right external jugular vein were cannulated for blood sampling and fluid replacement (with normal saline), respectively.

A 20 µl dose of the formulation was delivered to the right nostril only using a Hamilton syringe with attached length of polyethylene tubing, inserting the dosing tube about 0.5 cm into the nostril.

140 µl blood samples were collected in fluoride oxalate blood tubes (Sterilin Ltd., Middlesex, U.K.) and stored on crushed ice until analysis. Glucose determinations were carried out within 4 h of sampling using a Yellow Springs Instrument 23AM glucose analyser. Samples were taken 10, 6 and 2 min before nasal dose administration to establish baseline glucose levels and then 5, 10, 15, 20, 25, 30, 40, 50 and, if possible, 60 min after dosing.

Tissue Sampling and Processing

When required, the nasal tissue was fixed by cardiac perfusion of Bouin Hollandes solution after the collection of the final blood sample. The nasal cavity was then processed according to the methods described by Chandler et al., (10) to yield complete cross-sections of the nasal cavity.

Analysis of Data

The baseline blood glucose level was taken as the mean of the three pre-dose blood samples (−10, −6 and −2 minutes). The glucose concentration at each time point after dose administration was then expressed as % baseline concentration and plotted against time. The area under this curve (AUC) at 40 minutes was determined for each animal. Increased insulin absorption was signified by a decrease in AUC value. The rate of reduction of blood glucose levels was determined from the slope of ln % baseline concentration v time plots between 5 and 30 minutes, allowing for a lag time before onset of effect.

The effects of the lysophospholipid enhanced insulin formulations were compared with the previously reported control results obtained from the administration of insulin alone in phosphate buffer (100 IU/ml) (1).

Where appropriate, statistical analysis was carried out.

Single-factor analysis of varience (ANOVA) on the relevant AUC data determined the existence of any difference between the group means. To identify the source of any difference found (eg. between which treatment group and control) the Tukey method for multiple comparisons was employed (16, 17).

The histological effects of the formulations were determined by the analysis of cross-sections of the nasal cavity, randomly selected from each animal. The epithelium on the dosed side of the nasal septum was qualitatively compared with the tissue on the undosed side in the same section using the light microscope.

RESULTS

Insulin Absorption Enhancement Due to LPC Homologues

The fall in blood glucose levels, the rate of fall and the resulting AUC following the co-administration of insulin with individual LPC homologues are given in Table 1 together with control results previously obtained for the unenhanced insulin formulation (1).

Blood glucose levels were found to fall rapidly following the nasal administration of all LPC-enhanced insulin formulations except that containing LPC-caproyl. Differences in the absorption data (Table I) were generally small for lysophospholipids with fatty acid chain lengths of 10 carbon atoms or more. The slightly greater enhancing effect (smaller AUC) observed with homologues of intermediate chain length compared to the longer chain compounds may have been due to the slight increase in molar concentration as a result of dosing at the same % w/w.

The initial rate of fall in blood glucose levels appeared to be greater for LPC-oleoyl than LPC-stearoyl, but there was no significant difference in the overall effect of these two enhancers judging by the AUC data. For saturated and unsaturated 18-carbon fatty acid side-chains, any difference in molar concentration would have been negligible in this case.

The lysophospholipid, LPC-caproyl, with the shortest fatty acid chain (C6) of those investigated, resulted in no appreciable decrease of blood glucose levels when coadministered with insulin. In contrast, LPC decanoyl, with just a four-carbon extension of the fatty acid residue, produced a sharp fall in blood glucose levels.

These two enhancers were therefore chosen for further investigation in the combined absorption and histological model to determine whether this small structural change had similar contrasting effects on the nasal epithelium. They were administered at equimolar concentrations, calculated to correspond to the molar concentration of LPC-stearoyl previously used (11.9 mM). This would enable comparison of the three sets of data. The insulin dose was maintained at 8 IU/kg and tissue was fixed after the collection of the 40 minute sample. This allowed sufficient time for the determination of the blood glucose concentration profile without risking premature loss of animals by hypoglycaemia and meant that tissue was fixed at a time reasonably close to the time of actual drug absorption. The resulting absorption data for LPC-decanoyl and LPC-caproyl are given in Table II.

In this study, the inter-animal variation in the response to the nasal administration of the LPC-caproyl formulation was greater, as can be seen by the values of standard deviation in Table II.

Statistical analysis of the AUC data indicated that, despite increased variability in the LPC-caproyl results, the effects of equimolar concentrations of LPC homologues on blood glucose levels followed a similar pattern to that observed in the initial absorption experiments. LPC-decanoyl again resulted in a rapid fall in blood glucose levels and this was equivalent to that produced by LPC-stearoyl. Overall, the LPC-caproyl formulations resulted in no significant increase in effect compared to the control insulin formulation (at the 5% level).

The absorption enhancing effects of equimolar doses of LPC homologues with saturated fatty acid side chains of variable length were therefore summarised as follows:-

$$\text{LPC-stearoyl} = \text{LPC-decanoyl} > > \text{LPC-caproyl} = \text{No enhancer}$$

Comparison of the results for LPC-decanoyl at 15.2 mM and 11.9 mM in Tables I and II respectively, indicated that the enhancing effect of this LPC homologue decreased with con-

Table I. Summary of the Effects on Blood Glucose Levels of the Intranasal Administration of 8 IU/kg Insulin with a Range of LPC Homologues (0.625% w/v; 0.5 mg/kg) of Varying Fatty Acid Chain Length

		Rate of Fall (10^{-2} min^{-1})		% Fall (t = 40)		AUC (t = 0–40)	
Enhancer#	n	Mean	SD	Mean	SD	Mean	SD
—	6	—	—	−6.9	10.2	4132.7	348.8
LPC (16/18:0)	5	3.9	1.7	71.6	9.2	2487.1	171.9
LPC_s (18:0)	6	3.8	1.2	69.9	6.7	2126.0	275.5
LPC_o (18:1)	7	5.6	0.8	78.4	3.3	1927.6	203.1
LPC_l (12:0)	7*	7.1	1.6	82.7	10.0	1754.6	304.2
LPC_d (10:0)	6	6.0	1.2	82.5	3.5	1808.8	213.5
LPC_c (6:0)	7	—	—	4.8	6.8	3850.9	161.5

* Extrapolated data points used (n = 1–2).

Fatty acid chain description given in parentheses.

Number of carbons in side chain length: number of double bonds.

Table II. Summary of the Effects of LPC Homologues (11.9 mM) with Different Fatty Acid Chain Lengths on Blood Glucose Levels Following Intranasal Administration with 8 IU/kg Insulin

		Rate of Fall (10^{-2} min^{-1})		% Fall (t = 40)		AUC (t = 0–40)	
Enhancer#	n	Mean	SD	Mean	SD	Mean	SD
—	6	—	—	−6.9	10.2	4132.7	348.8
LPC_s (18:0)	6	3.8	1.2	69.9	6.7	2126.0	275.5
LPC_d (10:0)	8	3.7	1.5	67.8	12.0	2471.2	370.6
LPC_c (6:0)	6	−0.1*	0.9	4.6	35.0	4157.4	562.0

* Negative value implies an overall mean rate of rise.
# Fatty acid chain description given in parentheses.
Number of carbons in side chain length:number of double bonds.

centration. To further investigate the concentration dependence of LPC-decanoyl absorption enhancing activity this homologue was coadministered with 8 IU/kg insulin at a concentration of 5 mM (0.21 % w/v). Table III gives the effect of LPC-decanoyl on blood glucose levels at all three concentrations investigated.

At an enhancer concentration of 5.0 mM there appeared to be a further reduction in the effect on blood glucose levels, compared with the two higher concentrations. The same trend was observed in all of the absorption parameters calculated.

A one-way ANOVA test on the three sets of AUC results indicated that the difference in the effect with enhancer concentration, over 40 minutes, was significant (at the 5% level). Tukey analysis then identified that the reduction in blood glucose levels when 15.2 mM LPC-decanoyl was coadministered with 8 IU/kg insulin was significantly greater than with either of the two lower concentrations of enhancer, but that the difference between the effects of 11.9 mM and 5.0 mM was not significant.

Insulin Absorption Enhancement Due to LPG

Figure 2 shows the blood glucose profiles obtained following the nasal administration of insulin in combination with LPG at two concentrations (0.5% w/v and 0.0625% w/v). The calculated absorption data is given in Table IV.

Comparison of the data in Table IV indicates that over one hour there was no significant difference between the effects of the two LPG enhanced formulations on blood glucose levels despite an almost ten-fold difference in concentration.

Blood glucose concentration has fallen by 60% forty minutes after the nasal administration of both formulations which was slightly less than for the "active" LPC homologues after the same time. This may be accounted for by slight differences in the enhancer concentrations used, with a lower % w/v LPG administered in each case. Nevertheless, the dramatic reduction of blood glucose levels following the coadministration of 0.0625% w/v LPG with insulin indicates that this lysophospholipid is highly active as a nasal absorption enhancer.

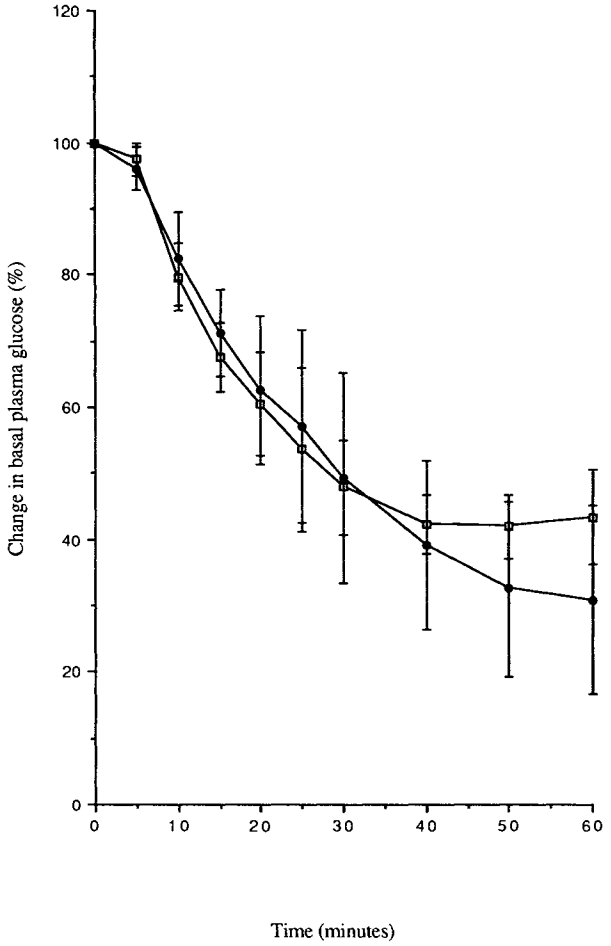

Figure 2: Plasma glucose levels following the intranasal administration to rats of 8 IU/kg insulin with LPG as enhancer at 0.0625% w/v (0.05 mg/kg) (●) and at 0.5% w/v (0.5 mg/kg) (□) (±SD).

Table III. Summary of the Effects of LPC-Decanoyl on Blood Glucose Levels Following Intranasal Administration with 8 IU/kg Insulin at Different Concentrations

LPC-decanoyl Concentration		Rate of Fall (10^{-2} min^{-1})		% Fall (t = 40)		AUC (t = 0–40)	
(mM)	n	Mean	SD	Mean	SD	Mean	SD
15.2	6	6.0	1.2	82.5	3.5	1808.8	213.5
11.9	8	3.7	1.5	67.8	12.0	2471.2	370.6
5.0	4	2.9	1.0	50.8	8.7	2711.8	192.8

Table IV. Summary of the Effects of LPG on Blood Glucose Levels Following Intranasal Administration with 8 IU/kg Insulin at Two Concentrations

LPC-decanoyl Concentration		Rate of Fall (10^{-2} min^{-1})		% Fall (t = 60)		AUC (t = 0–60)	
(% w/v)	n	Mean	SD	Mean	SD	Mean	SD
0.5	6	3.1	1.2	56.6	7.1	3461.9	241.0
0.0625	4	2.8	1.1	69.1	14.1	3334.3	642.8

Histological Effects of Lysophospholipid Enhanced Insulin Formulations

(a) Insulin 100 IU/ml with LPC-caproyl (11.9 mM)

LPC-caproyl resulted in few obvious interactions with the nasal epithelium (Figure 3a). There was a slight increase in mucus discharge and reduction in epithelial height on the septum of the dosed side compared to the control side, but there was no desquamation of the respiratory epithelium. No detached cells were observed, in the lumen of the cavity, and the epithelium over the turbinates was unaffected.

(b) Insulin 100 IU/ml with LPC-decanoyl (11.9 mM)

LPC-decanoyl also resulted in few changes to the nasal epithelium (Figure 3b). Again, there was an increase in the amount of mucus present in the lumen of the dosed side of the cavity compared to the undosed side, which, in some cases, extended to the dorsal meatus. There was some reduction in septal epithelium height and in a few areas nuclei were packed nearer the basement membrane, but no cells were lost, the cilia appeared unaffected and the close contact between cells was maintained.

There were increased signs of interaction at the junction with the keratinised stratified squamous epithelium, with disruption of the pseudostratified appearance of the respiratory epithelium, further mucus discharge and possible loss of cilia, but still no significant desquamation.

The histological effects of LPC-caproyl over 40 minutes were considered to be equivalent to those of the unenhanced insulin formulation (1). The reduction in epithelium thickness following contact with LPC-decanoyl was perhaps slightly greater but otherwise the effects were very similar to those of LPC-caproyl.

The effects of these LPC homologues on the nasal epithelium were therefore summarized as follows:-

$$\text{LPC-decanoyl} \geq \text{LPC-caproyl} = \text{No enhancer}$$

(c) Insulin 100 IU/ml with LPG (0.5% w/v)

The addition of 0.5% w/v LPG to the nasal formulation resulted in the discharge of large amounts of mucus and considerable cell loss from the septum and turbinates (Figure 4a). An epithelium was maintained over all surfaces, but was considerably reduced in height. Nuclei were concentrated

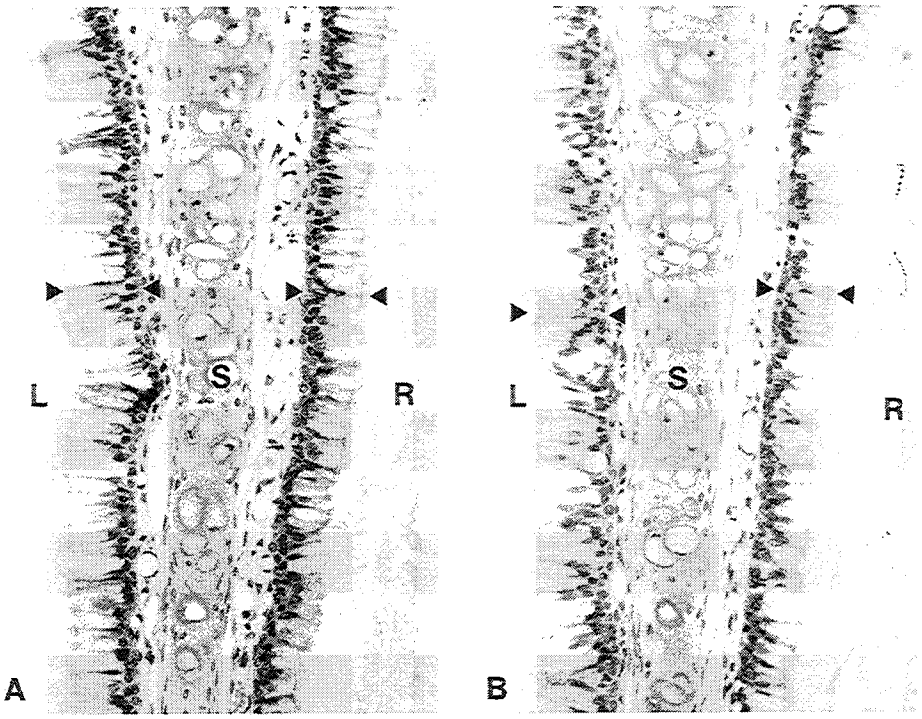

Figure 3: Photomicrographs of vertical sections through the anterior rat nasal cavity showing the two sides of the nasal septum (S) 60 min after dosing 20 µl of insulin solution (100 IU/ml) in phosphate buffer) into the right side (R), with the addition of absorption enhancers. Normal respiratory epithelium ie. ciliated pseudostratified columnar epithelium densely populated with pale staining goblet cell covers the undosed left side (L) in each case. Treated tissue on the right side can therefore be compared with untreated tissue on the left of the septum; epithelium thickness on the two sides is indicated by arrow heads. **(A)** LPC-Caproyl (11.9 mM, 0.42% w/v): There are few signs of epithelial interaction on the dosed right side of the nasal septum other than a slight increase in mucus discharge and reduction in epithelium thickness compared with the control left side. (HE.× 310). **(B)** LPC-Decanoyl (11.9 mM, 0.49% w/v): On the dosed side of the nasal septum there is some evidence of epithelial interaction. Compared to the untreated side there is an increase in the amount of mucus present in the lumen of the cavity, slight reduction in epithelial thickness and in a few areas nuclei are packed closer to the basement membrane. (He.× 310).

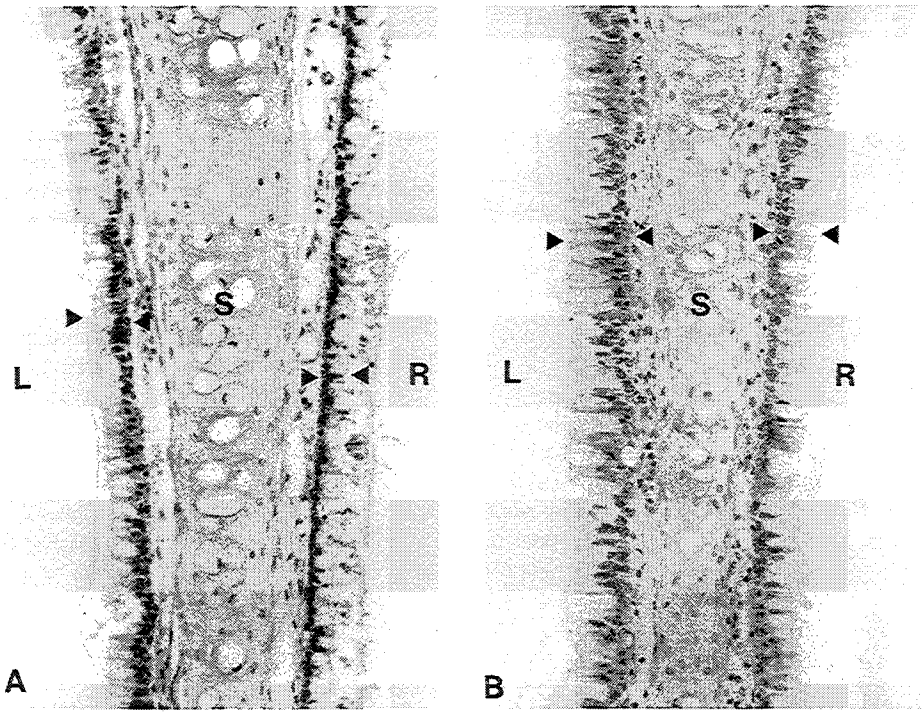

Figure 4: Photomicrographs of vertical sections through the anterior rat nasal cavity showing the two sides of the nasal septum (S) 60 min after dosing 20 μl of insulin solution (100 IU/ml) in phosphate buffer) into the right side (R), with the addition of absorption enhancers. Normal respiratory epithelium ie. ciliated pseudostratified columnar epithelium densely populated with pale staining goblet cells covers the undosed left side (L) in each case. Treated tissue on the right side can therefore be compared with untreated tissue on the left of the septum; epithelium thickness on the two sides is indicated by arrow heads. (A) LPG(0.5% w/v): The respiratory epithelium on the dosed right side of the nasal septum (S) has been disrupted. Lost cells are mixed with discharged mucus and nuclei in the remaining epithelium are packed towards the basement membrane. (HE. × 310). (B) LPG (0.0625% w/v): There are few signs of epithelium interaction with only a limited amount of mucus discharged into the lumen on the cavity on the dosed right side. The treated epithelium appears slightly reduced in thickness compared to the untreated tissue left side of the nasal septum (S). (HE. × 310).

towards the basal surface of the cells and in places the epithelium lacked cilia.

Respiratory infection was a problem in some of the animals during this part of the study. This was identified by signs of lymphocyte infiltration, mucus discharge and epithelial thickening in both sides of the nasal cavity. This highlighted another advantage of the "self-controlled" model. Non-experimental pathological changes in the control side of the nasal cavity may be identified and accounted for in comparison with the dosed side, or the animal excluded completely if the effects were severe. Such changes might have been mistaken for the effects of the test formulation in studies in which different animals were used as the test animal and the control animal. In this study, infection was generally localised and the effects of LPG easily distinguished in the dosed side of the nasal cavity.

(d) Insulin 100 IU/ml with LPG (0.0625% w/v)

The reduced concentration of LPG resulted in few signs of interaction with the nasal epithelium (Figure 4b). There was a slight increase in mucus discharged on the dosed side compared to the undosed side, but this was generally restricted to the septal regions only, without flow into the dorsal meatus. There was a slight reduction in epithelium thickness on the dosed septum with nuclei packed slightly closer to the basement membrane, but cilia remained intact and the pseudostratified epithelial structure was maintained in all areas.

The histological effects of 0.5% w/v LPG were considered to be comparable to those of the LPC (stearoyl/palmitoyl) preparation previously investigated (1). The LPG preparation used also had a mixture of fatty acid chain lengths, again primarily stearoyl and palmitoyl residues. The 0.0625% w/v LPG enhanced insulin formulation resulted in no significant change in nasal epithelial integrity and was again considered equivalent to the control insulin formulation in its histological activity.

DISCUSSION

In the present experiments lysophospholipid structure has been shown to affect absorption enhancing and histological activity. Care must be taken in the interpretation of absorption data using an indirect method such as the effect on blood glucose levels rather than actual insulin concentra-

tions, but gross differences are quickly and easily identified in this way.

A change in the degree of unsaturation within lysophospholipids which have long-chain fatty acid residues did not significantly alter efficacy, whereas the length of the fatty acid side-chain had profound effects on the activity of the compound. A minimum fatty acid chain length, greater than 6 carbons, was required for an enhancing effect to be exerted. The change in the nature of the polar head group of LPG while maintaining a long-chain fatty acid residue did not result in a dramatic change in enhancing effect at higher concentrations, but the similar response obtained using a very low concentration (compared to the fall off in response with concentration using LPC-decanoyl) may suggest that the head group may also have an influence on the enhancing efficiency; further studies would be required to confirm this.

A similar study has been reported in which the relationship between the absorption enhancing activity and membrane perturbing effects of acylcarnitines with differing saturated chain lengths was investigated (18). Acylcarnitines are similar to lysolecithins in having a polar head group (carnitine) of a similar size and nature to the choline-phosphate ester group of LPCs (19), which is also attached to a fatty acid moiety.

The polar antibiotic sodium cefoxitin was used as the model drug and the brush border membrane of the rat small intestine as the model membrane surface, assessed for integrity by a fluorescence polarization method. Acylcarnitines with fatty acid chains less than 12 carbon units in length were found to be ineffective in increasing drug absorption from the rat rectum *in vivo* and also in perturbing the order of the brush border membranes *in vitro*. The ability of the longer chain acylcarnitines (12-18 carbons) to enhance drug absorption generally correlated well with membrane perturbing activity with maximum activity observed with palmitoylcarnitine (16 carbons).

LeCluyse et al. (18) concluded that acylcarnitines induced absorption enhancement *in vivo* by interaction with biological membranes. In particular, it was suggested that a critical chain length (10 carbons) had to be surpassed for the acylcarnitines to partition into the membrane and also that the lipid order had to be disrupted beyond a threshold value (15-20%) before absorption enhancement could occur.

This report makes an interesting comparison for the present results. In a similar way, the LPC homologue with only 6 carbon units was an ineffective absorption enhancer, but LPC-decanoyl with a fatty acid residue of 10 carbon units resulted in rapid insulin absorption, judging by blood glucose levels. The variation in critical side-chain length for absorption enhancement may reflect the difference between LPC and acylcarnitines, or possibly differences in the nature of the nasal and rectal membranes involved.

The LPC-caproyl enhanced formulation resulted in few signs of membrane interaction in the rat nasal cavity and was considered comparable in histological effect to the control insulin formulation. This might have been expected considering the simultaneous lack of insulin absorption enhancement.

However, the membrane disruption resulting from exposure to the LPC-decanoyl enhanced insulin formulation was only marginally increased, in contrast to its significant effect on blood glucose levels. Furthermore, the histological effects of the LPC-decanoyl formulation were considerably less damaging than those observed following exposure to the original LPC formulation at a similar concentration and which resulted in a similar effect on blood glucose concentration.

This contrasts with the results of LeCluyse et al., (18), in that the absorption activity of LPC-decanoyl does not correlate well with observed membrane effects. Histological examination using the light microscope however, cannot be as sensitive as that of fluorescence polarization. Membrane interactions beyond a threshold value may also have occurred with LPC-decanoyl, but this may be a more subtle effect not easily distinguished at the level of examination used in this study.

It can be assumed that the histological effects of the 5.0 mM LPC-decanoyl enhanced insulin formation would be less than or equal to the 11.9 mM formulation investigated. The absorption enhancement at this lower concentration, although based on a more limited amount of data, was considered to be equivalent to that at 11.9 mM over 40 minutes. However, the effect of the two concentrations of LPC-decanoyl may not be equivalent over a longer time period; blood glucose levels were still showing a downward trend at 40 minutes following the coadministration of 11.9 mM LPC-decanoyl with insulin, but were levelling off or even starting to rise at the lower enhancer concentration. The histological effects of the 5.0 mM and also the 15.2 mM LPC-decanoyl enhanced formulations would be of further interest to establish whether the changes in absorption enhancing effect with concentration were related to membrane interactions. Nevertheless, the indication is that severe membrane disruption is not a prerequisite for the promotion of insulin absorption across the nasal mucosa.

These results were supported by the effects observed following the administration of LPG enhanced formulations. At the even lower concentration of 0.0625% w/v (approx. 1.2 mM) LPG was found to enhance insulin absorption to a similar extent as 0.5% w/v LPG and the active LPC homologues with few membrane damaging side effects. This suggests that careful selection of enhancer concentration may provide an adequate level of absorption enhancement with minimal disruption of the nasal epithelium.

Karlquist et al (20) found that permeability of rat stomach to different sized polyethylene glycols increased in the presence of taurodeoxychloate (2.5 and 5 mM) though to a lesser extent than LPC-stearoyl/palmitoyl, whereas the more hydrophilic taurocholate had no effect at the concentrations investigated. The increase in permeability was considered to be related to the ability of the different agents to interact with surface mucus and the lipid portions of the exposed membrane.

Variations in hydrocarbon chain length and degree of saturation similarly affect the hydrophilic nature of the lysophospholipid molecules and thus their surface activity and likely interactions with phospholipid membranes. The present results suggest that, as with acylcarnitines, if the lysophospholipid molecule is too hydrophilic as a result of a short fatty acid chain length, interactions with nasal membranes are not sufficient to result in increased permeability to large molecules such as insulin. A decrease in hydrophilic-

ity due to an increased hydrophobic side chain length will increase membrane interactions as shown by LPC-stearoyl-palmitoyl, but the level of interaction required to enhance absorption is less than that which results in severe membrane damage, as demonstrated by LPC-decanoyl. However, use of more hydrophobic compounds at lower concentrations may have similar results as demonstrated by LPG-stearoyl/palmitoyl.

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