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Effect of polymers and microspheres on the nasal absorption of insulin in rats

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Summary

Dextran microspheres and polymer solutions have been evaluated as potential vehicles for nasal administration of insulin in rats. The polymer solutions were either viscous (polyacrylic acid and sodium hyaluronate) or showed thermal gelation (poly-*N*-isopropylacrylamide and ethyl(hydroxyethyl)cellulose). The spheres were epichlorohydrin cross-linked dextran, Sephadex and DEAE-Sephadex. Administration of insulin at 1 IU/kg caused a significant decrease in plasma glucose level with two of the investigated polymer systems, polyacrylic acid and ethyl(hydroxyethyl)cellulose, and with the Sephadex spheres. Sodium hyaluronate and poly-*N*-isopropylacrylamide exerted a significant influence on the plasma glucose level when used as vehicle for an insulin dose of 5 IU/kg. Insulin in DEAE-Sephadex had no effect at all on the plasma glucose level. A larger reduction in plasma glucose level was observed with insulin carried in the particle system than in the polymer systems. Powder formulations which take up water and swell appear to be more efficient in promoting absorption of insulin than the polymer systems.

Introduction

The nasal administration of peptides has attracted much interest because of the relatively rapid absorption of drug with little metabolic degradation and the relative ease of administration. However, bioavailability is rather low for large molecules with this form of administration (Fischer et al., 1987). To overcome this problem, it is possible to use dosage forms which remain in the nasal cavity long enough to ensure effective absorption. Viscous polymer solutions increase the contact time between the drug and the nasal mucosa and thereby increase the bioavailability (Morimoto et al., 1985). Enhancer systems which affect the mucosal cell barrier and mucociliary clearance have been investigated, for example, surfactants (Hirai et al., 1981b), bile acid salts (Duchateau et al., 1986), fatty acids (Mishima et al., 1987) and bile acid salts in combination with fatty acids (Tengamnuay and Mitra, 1990). However, although these systems promote the bioavailability of peptide drugs, ciliatoxicity and damage to the nasal mucosa have been noted. Bile acid salts and non-ionic surfactants, in particular, are associated with toxic effects on the

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nasal mucosa (Martin et al., 1978; Hirai et al., 1981c).

Increased absorption of insulin has been observed when using a powder formulation which is insoluble in water but which absorbs water, e.g., degradable starch microspheres (DSM) (Björk and Edman, 1988). The fact that the spheres remain in the nasal cavity for a long time (Illum et al., 1987) was thought to facilitate the absorption of insulin. However, Björk and Edman (1990) speculated that the enhanced absorption of insulin mediated by DSM was not solely due to prolonged contact time. They suggested that the uptake of water by DSM and subsequent swelling might cause dehydration of the epithelial cells, leading to widening of tight junctions and thereby facilitated paracellular transport of large hydrophilic molecules. When DSM is combined with a biological enhancer, lysophosphatidylcholine (LPC), the extent of absorption is improved even further (Farraj et al., 1990).

In this paper, the efficacy of two particle systems and four polymer solutions in promoting nasal absorption of insulin are evaluated. The particle systems are based on solid epichlorohydrine cross-linked dextran spheres: Sephadex and DEAE-Sephadex. DEAE-Sephadex is an anion-exchange resin which consists of dextran cross-linked with epichlorohydrine and substituted with 2-ethylaminoethyl groups.

The rationale behind the nasal use of Sephadex and DEAE-Sephadex is to ascertain whether the effect with these spheres is similar to that seen with degradable starch microspheres (DSM), in order to develop a theory of general particle/powder effect, irrespective of the material in the spheres. DEAE-Sephadex is included in this study to investigate whether controlled release of insulin can be achieved using the ion-exchange principle.

The polymer systems were chosen from two standpoints, either slightly viscous systems or those that are thermoreversible, i.e., in which the viscosity depends on whether the temperature is below or above the lower critical solution temperature (LCST). Some acrylamide derivatives have LCSTs at 32–34 °C, just below the temperature of the nasal cavity. Solutions of the nonionic cellulose ether ethyl(hydroxyethyl) cellulose also possess LCSTs which for the quality used in this study lie in the range of 30–32 °C. These polymer solutions have low viscosity, which facilitates administration at room temperature. When the temperature is raised above the LCST in the acrylamide solution a stiff gel is formed, instantly releasing water and water-soluble drugs (Hoffman et al., 1986). However, when poly-*N*-isopropylacrylamide is copolymerized with polyacrylamide, a highly viscous system with incorporated water can be formed at 32–34 °C, without phase separation, i.e., the LCST is now above this temperature range.

The ethyl(hydroxyethyl)cellulose (EHEC) system shows thermal gelation without phase separation when low concentrations of ionic surfactants are present (Carlsson et al., 1990). Binding of the surfactants to the polymer produces micelle-like aggregates along the polymer chain. Furthermore, an increase in temperature leads to stronger attraction between EHEC and the aggregates (Carlsson et al., 1989a,b). As a consequence, these aggregates can act as cross-links between different polymer chains, resulting in an increase in viscosity at elevated temperature (Carlsson et al., 1990).

Experimental

Materials

Sephadex G-25, DEAE-Sephadex A-25, sodium hyaluronate (Mol. Wt 7.6×10^6 g/mol), polyacrylic acid (Mol. Wt 3×10^6 g/mol) (Carbopol 934P) and poly-*N*-isopropylacrylamide copolymerized with 5% polyacrylamide (PNiPAAm co-PAAm) were obtained as gifts from Kabi Pharmacia, Uppsala, Sweden. Ethyl (hydroxyethyl)cellulose (EHEC) of medical grade was obtained from Berol Nobel, Stenungsund, Sweden. Human monocomponent insulin (100 IU/ml) was purchased from Novo Denmark. All other chemicals were of analytical grade.

Preparation of gels

Sodium hyaluronate, polyacrylic acid and PNiPAAm co-PAAm were presoaked overnight in

physiological saline. Polyacrylic acid was neutralized with 10% sodium hydroxide to achieve an increase in viscosity. A pH between 4.5 and 7.5 in the polyacrylic gel does not influence the intranasal absorption of insulin, according to Morimoto and co-workers (1985). Insulin was added to give 10 and 50 IU/ml and the final polymer concentration was adjusted to 0.075% (w/v) sodium hyaluronate, 0.5% (w/v) polyacrylic acid and 1% (w/v) poly-*N*-isopropylacrylamide. A 1% (w/w) solution of EHEC in water with 3.0 mmolal sodium dodecyl sulfate (SDS) was prepared. The solution was kept in a refrigerator for 1 week to ensure complete dissolution (Carlsson et al., 1990). Insulin and water were added to give a concentration of 0.8% EHEC and 2.4 mmolal SDS, and an insulin activity of 10 IU/ml.

Preparation of spheres

Sephadex was mixed with insulin (100 IU/ml) in a proportion of 100 mg spheres per ml insulin solution. The suspension was then freeze-dried. The dry powder was passed through a 180 μm sieve.

DEAE-Sephadex was dispersed in a buffer solution according to the manufacturer's instructions to equilibrate the ion-exchange groups. After sedimentation, the gel thus formed was mixed with insulin and buffer in the same proportions as for the Sephadex particles. Again the suspension was allowed to sediment and the gel was freeze-dried. The dried spheres were passed through a 180 μm sieve.

In vitro release

10 mg of the spheres or 0.5 ml of the polymer preparations were placed in a diffusion chamber according to the method outlined by Björk and Edman (1990). The receiving compartment contained 0.15 or 0.86 M sodium chloride solution. The chamber was immersed in a water bath at 37 °C, and samples of 100 μl were withdrawn 3, 5, 10, 15, 30, 60, 120 and 180 min after the experiment started. Each sample was replaced with an equal volume of sodium chloride solution.

Animal experiments

Rats have been widely used for intranasal drug delivery studies. Three main models, with minor modifications, have been described (Gizurarson, 1990). The model used in these experiments is a modification of the in vivo surgical model. Instead of preventing the drainage of drug from the nasal cavity, by sealing the nasopalatine tract leading from the nasal to the oral cavity with an adhesive glue (Hussain et al., 1980), normal function was maintained to the fullest possible extent.

Male Sprague Dawley rats (Alab AB, Sweden) weighing 200–250 g were fasted for 15–17 h prior to the experiments. Anaesthesia and surgery were performed as reported earlier (Björk and Edman, 1988). After an intraperitoneal injection of thiobutabarbital sodium (Inactin, BYK), 120 mg/kg, the rats were placed in a supine position on heated plates to maintain their body temperature. The trachea and the arteria carotis were cannulated with polyethylene tubes. The insulin preparations were administered through the nostril using a polyethylene tube 30 min post-operation. The spheres were weighed into the tube to give a dose of 1 IU/kg and an amount of 5 mg dry powder/kg in accordance with the optimization studies performed by Björk and Edman (1990). The spheres were administered by blowing air from a syringe through the tube. The viscous solutions were administered by volume, 20–25 μl, to give doses of 1 and 5 IU/kg. Blood samples of 150 μl were withdrawn from the arteria carotis 10, 20, 30, 40, 50, 60, 120, 180 and 240 min after administration. After centrifugation, the plasma was withdrawn and frozen for glucose analysis.

Analysis

The protein content in the in vitro release study was determined by assaying Folin according to Lowry et al. (1951).

The plasma glucose levels were measured using hexokinase and glucose-6-phosphate dehydrogenase (Beckman Dri-STAT). Insulin activity in the formulations was determined by radioimmunoassay (RIA, Pharmacia Insulin RIA 100).

Calculation

The area under the change in plasma glucose vs time curves (AUC) was calculated according to

the trapezoidal rule. The changes in plasma glucose were in the range of 0–100% and the time span was from 0 to 4 h. The total decrease in plasma glucose level ($D\%$) was calculated as stated by Hirai et al. (1981a):

$$D\% = \frac{AUC_c - AUC_{in}}{AUC_c} \times 100$$

where AUC_c and AUC_{in} denote the area under the curve for intranasal administration of insulin 1 IU/kg in physiological saline (control) and for different intranasal administration systems for insulin, respectively.

Statistical significance was tested using one-way ANOVA followed by the Student-Newman-Keuls test.

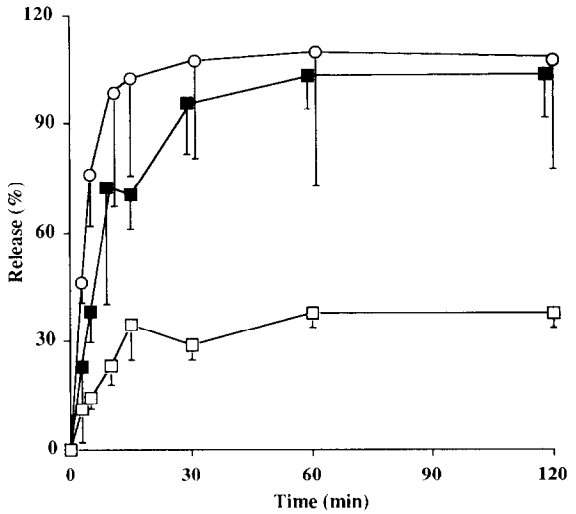

Fig. 1. In vitro release profile for insulin from dextran particle systems into different receiving media. Each point represents the mean and the standard deviation (S.D.) of three experiments. (○) Sephadex/0.15 M sodium chloride; (□) DEAE-Sephadex/0.15 M sodium chloride and (■) DEAE-Sephadex/0.86 M sodium chloride.

Results and Discussion

The release kinetics of insulin from the two particle systems are shown in Fig. 1. The release rate of insulin from Sephadex is the same as that for the degradable starch microspheres (DSM) used by Björk and Edman (1990). Both the starch microspheres and the dextran particles release 90% of the incorporated insulin within 10 min.

This is no surprise, since the swelling factor of the dextran particles (4–6) is more or less equal to that for the degradable starch microspheres (5) used by Björk and Edman, (1990). The swelling factor, defined as the bead volume in cm^3 obtained when 1 g microspheres is allowed to swell in a buffer, is low when the degree of cross-linking is high. Spheres with a low swelling factor need less liquid in order to swell completely, and the release of insulin will be rapid.

DSM have a very narrow particle size distribution, with a mean particle size of 45 μm, whereas the Sephadex particles are between 50 and 180 μm in diameter. The wide range in particle size distribution may be the cause of the large variation observed for the Sephadex system in this in vitro study.

DEAE-Sephadex requires a high concentration of saline in the receiving compartment before the insulin is released. This implies that the insulin is bound to the DEAE groups and can only be displaced in solutions of high ionic strength. The results are contradictory to the findings of Manosroi and Baur (1990). They predicted a hydrophobic, not an ionic, interaction between insulin and DEAE-dextran. However, their conclusion was drawn from data obtained at lower saline concentrations than the 0.86 M sodium chloride used in the present experiments.

Insulin (1 IU/kg body weight), administered intranasally with Sephadex particles, induced a rapid decrease in plasma glucose, but when administered with DEAE-Sephadex no effect was observed on the glucose level (Fig. 2). These findings correlate well with the in vitro release results. Sephadex particles release the insulin rapidly and maximal lowering, by approx. 25%, of the plasma glucose was attained 40–60 min after administration. The decrease in plasma glucose is significantly different ($p < 0.05$) from that seen with placebo and the control (insulin 1 IU/kg in

physiological saline) at 20, 30, 40 and 60 min after administration. No significant difference was seen at 50 min due to a large standard deviation in this point. The large variation observed can be predicted from the in vitro results. A considerable range in release rate measurements, due to the wide distribution of particle sizes, also causes great variation in the absorption of insulin.

The enhanced absorption of insulin in the powder formulation might be due to an effect on tight junctions between the epithelial cells. In order to exert such an effect, the powder must absorb water and yet be water-insoluble (Björk and Edman, 1990). Both Sephadex and DSM meet these criteria. Both particle systems provide rapid in vitro release of insulin and a rapid decrease in plasma glucose. No sustained release is observed, supporting the hypothesis that the increased residence time of the insulin formulation in the nasal cavity is probably not the only parameter governing the observed increase in absorption.

On the other hand, insulin in the DEAE-Sephadex system exhibited no effect on the plasma glucose level. A possible explanation may be that

TABLE 1

Total decrease (D%) and maximal decrease of plasma glucose level after intranasal administration of insulin in different vehicles

	Dose (IU /kg)	*D* (%)	Maximal decrease (%)	*n* (number of animals)
Control	1		5 (2.8)	5
	5	1.9 (2.9)	7 (4.2)	5
Sodium hyaluronate	1	1.6 (0.4)	8 (1.9)	3
	5	10.5 (5.5)	22 (5.9)	3
Polyacrylic acid	1	8.3 (3.6)	19 (3.9)	5
	5	10.0 (6.6)	18 (4.8)	5
PNiPAAm co-PAAm	1	2.0 (2.8)	8 (4.2)	3
	5	11.9 (6.2)	20 (6.8)	3
EHEC-SDS	1	4.2 (3.1)	12 (2.0)	4
Sephadex	1	8.4 (5.6)	25 (18.2)	3
DEAE-Sephadex	1	4.8 (6.8)	9 (6.2)	3
DEAE-Sephadex and 0.86 M NaCl	1	0.1 (0.2)	6 (1.2)	2

Data are expressed as means with the standard deviations (S.D.) indicated in parentheses.

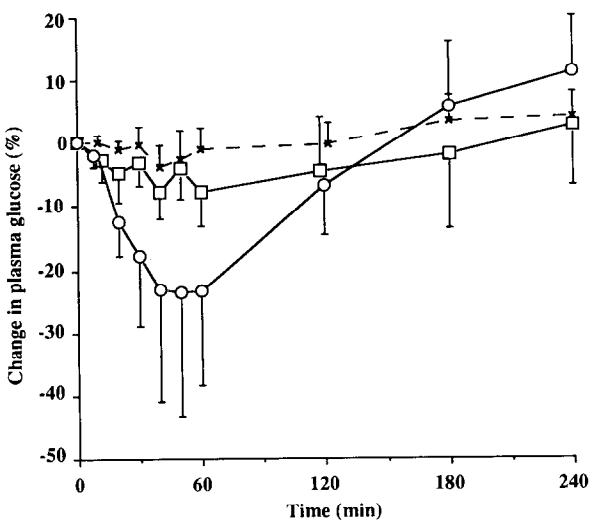

Fig. 2. Hypoglycaemic response after intranasal administration of insulin 1 IU/kg in two dextran particle systems, Sephadex and DEAE-Sephadex. Data are expressed as means ±S.D. (○) Sephadex, (□) DEAE-Sephadex and (×) control; 1 IU insulin per kg in physiological saline.

the insulin is not displaced from the DEAE groups due to the low electrolyte concentration in the nasal cavity. Saline of high concentration (0.86 M) was therefore added 5 min after administration of the powder, in order to displace the insulin, however, the lack of a decrease in plasma glucose was still observed (Table 1). This indicates that insulin must be available for absorption at the same time as the spheres swell and affect the superficial epithelial cells. The later release of insulin, after the swelling process of the spheres has reached completion, has no effect on the plasma glucose level. This indicates that withdrawal of water from mucus, and probably also from underlying cells, is of major importance to achieve enhanced absorption of insulin from the vehicle system involving spheres.

The polymer systems, in general, release insulin more slowly than the Sephadex particles (Fig. 3). Approx. 60 min is needed for the viscous

systems, polyacrylic acid and sodium hyaluronate solutions, to reach an accumulated release of 90%. The release rate is even slower with the thermoreversible polymers. The EHEC system releases 90% of its insulin content within 90 min, while for the PNiPAAm co-PAAm system 150 min is needed to release the same amount of insulin.

Insulin was administered to rats at doses of 1 and 5 IU/kg in polyacrylic acid (Fig. 4B), sodium hyaluronate (Fig. 4A) and PNiPAAm co-PAAm (Fig. 5A). It was only possible to incorporate 10 IU insulin per ml in the EHEC system and therefore results are available only for the dose of 1 IU/kg (Fig. 5B).

The insulin dose of 1 IU/kg in one viscous polymer solution, polyacrylic acid, and one thermoreversible polymer solution, EHEC, had a significant effect ($p < 0.05$) on the blood glucose level.

Insulin in the EHEC system caused a rapid decrease in blood glucose level, with the maximal effect being reached after approx. 40 min. The decrease was significant from 20 min up to 1 h after administration, however, the plasma glucose level was only reduced by 12% (Fig. 5B). In the EHEC system, a small amount of ionic surfactant, SDS, was present. In this case, the surfactant is bound to the polymer (Carlsson et al., 1989a,b), i.e., the concentration of free, non-bound SDS in the gel system is low. It has also been shown in an in vitro study that the release of ionic surfactant from the gel system is quite slow (Lindéll et al., 1991). It is therefore not likely that the positive influence observed on insulin absorption is related to any effect of free surfactant on the mucosal cell barrier. However, it should be borne in mind that Anderberg and co-workers (1992) have demonstrated that very low concentrations of SDS affect the integrity of monolayers of human intestinal epithelial (CaCo-2) cells.

For the polyacrylic acid solution (Fig. 4B), the decrease in plasma glucose was only significant between 40 and 60 min after administration. This system showed no dose dependency. When insulin 5 IU/kg was administered the plasma glucose was lowered to the same level as with 1 IU/kg. The reason for the lack of dose dependency is unclear. A reduction of 18–19% occurred from 50 to 60 min after administration with both doses. However, the decrease was significantly different from the control for a longer period, from 20 to 60 min after administration, for the higher dose.

Only the 5 IU/kg dose of insulin resulted in an effect on the plasma glucose level with the sodium hyaluronate (Fig. 4A) and thermoreversible PNiPAAm co-PAAm (Fig. 5A) systems. The effect with 1 IU/kg was not significantly different from that of the control. Insulin (5 IU/kg body weight) given with sodium hyaluronate causes a significant reduction in the plasma glucose level ($p < 0.05$) between 10 and 60 min after administration. Maximal decrease (22%) was achieved 40 min post-dosing. The acrylate system was comparatively slower in affecting the glucose level than the sodium hyaluronate. The effect was significantly different from the control between 40 and 180 min. The slightly delayed onset and the prolonged effect observed

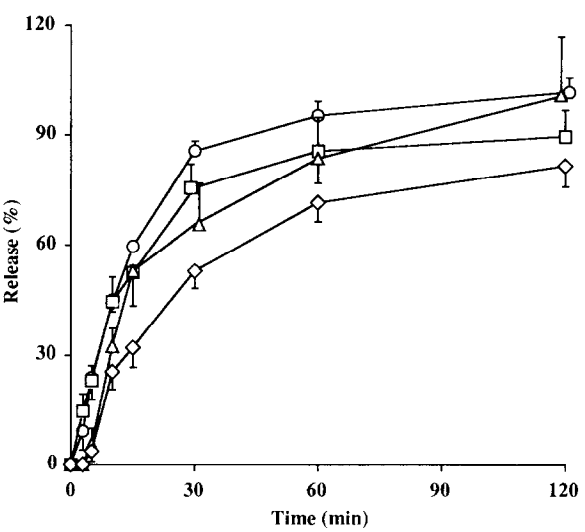

Fig. 3. In vitro release profile for insulin from polymer systems. Each point represents the mean and S.D. of three experiments. (○) 0.075% sodium hyaluronate, (□) 0.5% polyacrylic acid, (△) 0.8% ethyl(hydroxyethyl)cellulose/2.4 mmolal SDS and (◇) 1.0% poly-*N*-isopropylacrylamide co-polyacrylamide.

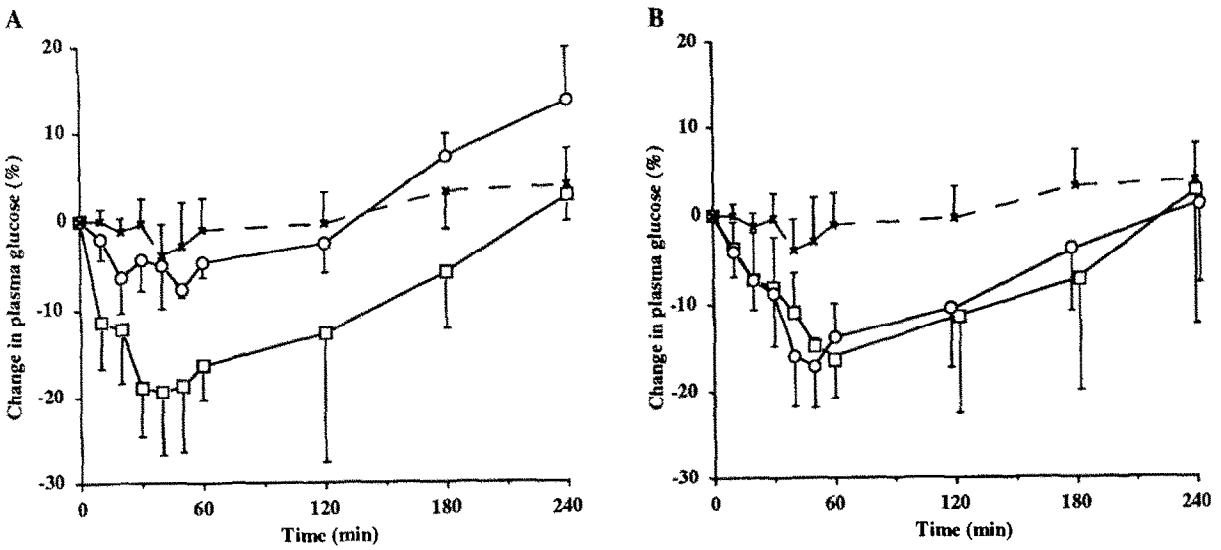

Fig. 4. Change in plasma glucose level (mean ± S.D.) after intranasal administration of insulin, (○) 1 and (□) 5 IU/kg, in viscous polymer systems and (×) control: 1 IU/kg insulin in physiological saline. (A) 0.075% sodium hyaluronate; (B) 0.5% polyacrylic acid.

with the latter system can be predicted from the in vitro release data. However, the time to reach the maximal decrease in plasma glucose level was not very different from the other systems tested, i.e., approx. 50 min after administration. The in vivo results are summarized in Table 1.

Polymers such as acrylates and natural macromolecules, for example, sodium hyaluronate, are mucoadhesive (Leung and Robinson, 1988; Saettoni et al., 1989). Evidently, when these polymers are given with drugs, they affect clearance from the nasal activity and create conditions which increase contact time.

A recent paper by Morimoto et al. (1991) demonstrates the use of sodium hyaluronate solution as a vehicle for intranasal administration of vasopressin and a vasopressin analogue. They report increased absorption of vasopressin when administered with sodium hyaluronate. The effect was correlated to the molecular weight and concentration of the polymer. Furthermore, mucoadhesion occurred and no cilia toxicity was seen with sodium hyaluronate. Considering the above study and the results of the present investigation with insulin, it is clear that sodium hyaluronate of high molecular weight might possess advantages as a biocompatible vehicle for drugs given nasally. The mechanism of action of sodium hyaluronate is unclear. That the prolonged contact time between drug and mucosa is due to the high viscosity and the mucoadhesion of the hyaluronate solution is obvious, but the effect of the molecular weight and concentration of sodium hyaluronate is interesting.

A comparison between polymer systems (both viscous and thermoreversible) and powder systems (Sephadex and DSM) shows clearly that the particle systems are more effective. A rapid onset and efficacious biological effect are observed. However, the efficacy of insulin plus Sephadex particles in lowering plasma glucose is inferior to that of DSM at equivalent doses. The reasons for this may involve discrepancies in size, and the location of the insulin, either inside or at the surface of the spheres. The latter parameter is dependent on the cut-off limit of the spheres. DSM is much more porous whereas Sephadex is considerably denser.

In summary, the results from this paper indicate that it is possible to achieve enhanced absorption of insulin using different formulations, e.g., dry powders which swell and viscous gel

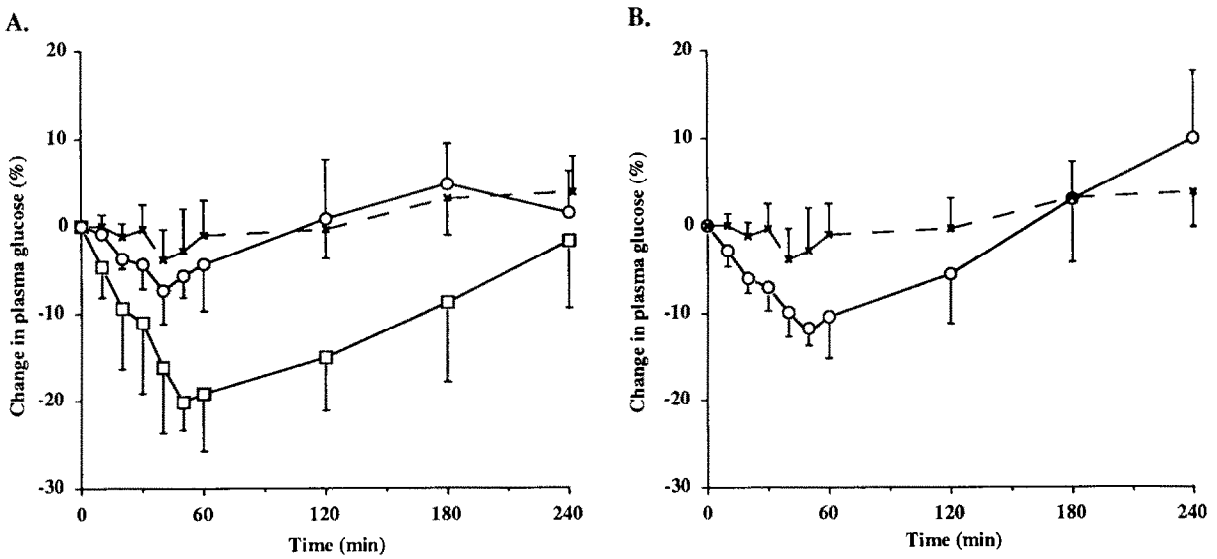

Fig. 5. Change in plasma glucose level (mean ± S.D.) after intranasal administration of insulin, (○) 1 and (□) 5 IU/kg, in thermoreversible polymer systems and (×) control: 1 IU/kg insulin in physiological saline. (A) 1.0% poly-*N*-isopropylacrylamide co-acrylamide; (B) 0.8% ethyl(hydroxyethyl)cellulose/2.4 mmolal SDS.

systems. Despite the differences in release observed in vitro, the maximal effect is reached 40–50 min after administration for all systems. The release rate of insulin obviously does not influence the kinetics of the effect vs time curves. An explanation for this phenomenon may be that both the swellable powder and the polymer gels affect the tight junctions between the epithelial cells, increasing the paracellular transport of hydrophilic insulin.

However, the maximal decrease in plasma glucose appears to be affected by the in vitro release rate. At equal doses of insulin (1 IU/kg), the fastest releasing system, Sephadex, causes the largest decrease in plasma glucose whereas the slowest releasing PNiPAAm polymer produces the least effect. The explanation might be that when insulin is administered in a slow releasing polymer system, there is little insulin available for absorption during the initial phase when the tight junctions might be affected.

The sodium hyaluronate system does not follow this trend. This is the fastest releasing polymer system, however, no significant decrease in plasma glucose is still observed. The reason for the lack of effect is unclear.

Increased residence time for the formulation in the nasal cavity is also a parameter which offers possibilities for improved drug absorption. The slower release of insulin from a mucoadhesive polymer, such as PNiPAAm co-PAAm, results in a prolonged effect. However, to achieve rapid absorption of insulin, thereby simulating the physiological profile for the endogenous secretion of insulin, a direct effect on the mucosal barrier is necessary, resulting in the immediate uptake of insulin. This objective can be attained by using dry spheres and maybe also with polymer gels which release insulin rapidly.

Further characterization of dextran spheres (Sephadex) is required before they can be evaluated as a potential nasal drug delivery system. The influence of particle size and cut-off limits should be investigated and the toxic effect on cilia must be examined. It would also be interesting to investigate whether the mechanism for the

improved absorption seen with the thermoreversible polymers is similar to the model of action for dry powder formulations.

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