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Report

Bile Salt–Fatty Acid Mixed Micelles as Nasal Absorption Promoters of Peptides. II. *In Vivo* Nasal Absorption of Insulin in Rats and Effects of Mixed Micelles on the Morphological Integrity of the Nasal Mucosa

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The effectiveness of mixed micelles in promoting nasal absorption of peptides has been demonstrated *in vivo* by employing insulin as a model compound. Insulin in the absence of adjuvants was not absorbed following intranasal administration. The results confirmed previous findings by others that absorption of insulin via alternative routes required absorption enhancers. Mixed micelles between NaGC and linoleic acid were found to rapidly deliver insulin into systemic circulation, with a concomitant decrease in plasma glucose. The extent of the hypoglycemic response was significantly greater than that produced by NaGC alone (55 vs 47%, $P < 0.05$). Emulsion of linoleic acid, on the other hand, did not produce any significant insulin absorption. The findings thus supported previous *in situ* data that mixed micelles were more effective than NaGC or linoleic acid in promoting nasal absorption of peptides. Histopathologic examination of the rat nasal mucosa revealed that the extent of morphological alterations caused by mixed micelles was of mild to moderate severity even after 5 hr of exposure. However, studies involving more frequent and prolonged exposures are necessary to assess the practicality of these adjuvants before any clinical application can be attempted.

KEY WORDS: mixed micelles; insulin, nasal absorption, hypoglycemic effect; sodium glycocholate; linoleic acid.

INTRODUCTION

In our previous report, it has been demonstrated that mixed micelles composed of bile salt (NaGC) and unsaturated fatty acid (linoleic acid) were effective in promoting nasal absorption of peptides such as [D-Arg^2]kyotorphin *in situ* (1). The adjuvant activity was significantly greater than that of bile salt or lipid alone. In addition, the promoting effect appeared to be reversible since the nasal membrane permeability returned to its original impermeable state within 20–40 min following removal of the mixed micelles from the rat nasal cavity. The question remains as to whether these adjuvants will be effective *in vivo*.

Insulin has been chosen as a model polypeptide in this study for two reasons. First, this peptide is among the most widely studied for possible absorption through alternative routes, particularly the nasal route. Approximate comparisons between the results obtained in this study and other reported results would be possible. Second, improved methods of insulin delivery could significantly influence diabetes treatment. The life-long existence of diabetes has made the parenteral route extremely inconvenient, particularly for elderly and juvenile patients who are unable to self-administer the drug routinely. This has led to the development of nonparenteral approaches to insulin therapy. Among the various nonparenteral routes investigated, the rectal and nasal routes appear to be the most effective (2). However, even with these two routes, the use of certain types of absorption promoting agents seems necessary to achieve significant absorption. Incomplete absorption is probably due to the poor membrane permeability of these mucosae and/or to metabolism at the absorption site (2).

Among several compounds employed as nasal absorption promoters, bile salts are the most studied in both animals and humans (3–8). In general, based on scanning electron microscopic observations, bile salts are less irritative to the nasal mucosa than synthetic surfactants such as polyoxyethylene 9 lauryl ether and are reported to be well tolerated by animals and human subjects, at least during a short term treatment (4,8). Furthermore, Gordon *et al.* (3) found a positive correlation between the hydrophobicity of bile salts and their adjuvant potency in promoting insulin nasal absorption. However, Duchateau *et al.* (9) reported that bile salts can have an inhibitory effect on the ciliary movement of the nasal mucosa. They found that ciliotoxicity increased with increasing hydrophobicity of the bile salts. In general, dihydroxy bile salts appear to be more toxic than trihydroxy

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bile salts, with deoxycholate being the most toxic. These findings are in agreement with Hersey and Jackson (10), who reported that sodium deoxycholate at 0.5% caused tissue damage in the dog nasal mucosa. In addition, solubility of the more hydrophobic bile salts might become a problem, particularly in the environment of the absorption site, and could result in the decreased adjuvant activity (9). Therefore, the use of bile salts has been restricted mainly to the more hydrophilic trihydroxy bile salts such as sodium glycocholate due to its mild effects on the nasal mucosa (8,11). Nevertheless, as demonstrated by Gordon *et al.* (3), sodium glycocholate was much less effective than deoxycholate in promoting insulin nasal absorption.

Hence, the purpose of this study was to demonstrate that mixed micelles consisting of sodium glycocholate and linoleic acid were effective in delivering insulin across the rat nasal mucosa into systemic circulation, with a concomitant hypoglycemic response, and to see if the enhancement was greater than single adjuvants. In addition, histology studies were performed to evaluate morphological integrity of the nasal mucosa in the presence of mixed micelles under various exposure times. The results obtained would partly address the feasibility of utilizing such an adjuvant combination in the development of nasal drug delivery systems.

MATERIALS AND METHODS

Crystalline porcine zinc insulin (Iletin) was obtained as a gift from Eli Lilly and Co. (Indianapolis, IN). The same lot number (009HC7) was used in all experiments. Sodium glycocholate (NaGC) and linoleic acid (both certified as being at least 99% pure) were purchased from Sigma Chemical Co. (St. Louis, MO). All compounds were used as received. Insulin activity was 25.0 U/mg. Solutions were prepared by dissolving an appropriate amount of insulin in a mixed micellar solution consisting of 15 m*M* NaGC and 5 m*M* linoleic acid in isotonic 0.07 *M* phosphate buffer, pH 7.4. The choice of this ratio of bile salt to fatty acid was taken as a starting point because our previous findings (1) found this composition to be optimal in enhancing nasal absorption of the dipeptide [D-Arg^2]kyotorphin. Preparation of the mixed micelles has also been previously described (1). In addition, insulin solutions without adjuvants and in 15 m*M* NaGC micellar solution or 5 m*M* linoleic acid emulsion were also prepared in the same buffer. Another control experiment was performed by intranasal administration of a mixed micellar solution containing 15 m*M* NaGC and 5 m*M* linoleic acid without any added insulin.

In Vivo *Absorption Experiments.* Male Sprague–Dawley rats weighing 250–300 g were fasted for at least 16 hr prior to the experiments but were allowed to drink water ad libitum. The surgical procedure for the *in vivo* absorption study was that described by Hirai *et al.* (12). The rats were anesthetized with sodium pentobarbital. After surgery, an insulin preparation was administered to the rat's nasal cavity, at a volume of 100 μl/300 g body weight, through one of the nostrils via a microsyringe which was attached to a blunt needle. The doses administered were 2 and 5 U/kg body weight. Therefore, to achieve the dose and administration volume specified above, the concentrations of insulin in the preparations were calculated to be 7.5 and 15 U/ml for 2 and 5 U/kg doses, respectively. Blood samples (0.3 ml each) were withdrawn via the jugular vein at 0, 15, 30, 60, 90, and 120 min following nasal administration. For comparison purposes, an intravenous insulin dose of 0.25 U/kg was administered and blood samples were taken at 0, 2, 5, 15, 30, 60, 90, and 120 min postadministration. Samples were collected in heparinized natelson capillary tubes (Scientific Products, Milwaukee, WI) and centrifuged at 3000 *g* for 15 min using a Beckman TJ-6 refrigerated centrifuge. Plasma samples were isolated and kept frozen at −20°C for analysis of both immunoreactive insulin and glucose.

Histological Studies. Histopathological examination of the rat nasal cavity was performed according to the method of Young (13). Similar surgical procedure was carried out in anesthetized rats as in the *in vivo* experiments. Solutions containing mixed micelles of 15 m*M* NaGC and 5 m*M* linoleic acid in isotonic 0.07 *M*, pH 7.4, phosphate buffer were administered through one of the nostrils at a volume of 100 μl/300 g body weight. After 1-, 3-, and 5-hr exposure periods, each rat was decapitated. The eyes and integument were removed from the head. The lower jaw, brain, and pituitary were also taken. The nasal cavity was then gently flushed with 10–15 ml of neutral phosphate-buffered formalin solution. The head was immediately placed in the same formalin solution and fixed for 1 week. Following fixation, the specimen was decalcified and processed in a conventional manner. For control experiments, one group of rats received only phosphate buffer, another was treated intranasally with 15 m*M* NaGC micellar solution for 3 hr, and the third group was treated with 5 U/kg of insulin and 15 m*M* NaGC micellar solution. Each treatment consisted of three rats to provide meaningful interpretation of the results. Four standard cross sections of the nasal cavity were prepared and stained with hematoxylin and eosin for subsequent examination under light microscope.

Plasma Insulin Determination. Plasma immunoreactive insulin was quantitated by a double-antibody radioimmunoassay using an RIA kit provided by Cambridge Medical Technolgy (Billerica, MA).

Plasma Glucose Determination. Plasma glucose levels following nasal administration of insulin were determined by a modified *O*-toluidine method using a kit commercially available from Sigma Chemical Co. (St. Louis, MO).

Data Analysis. The areas under the plasma insulin or glucose concentration versus time curves ($AUC_{insulin}$ or $AUC_{glucose}$) were calculated by a linear trapezoidal rule from 0 to 120 min. Since the absorption of insulin following adjuvant addition is extremely rapid (peak time of 15 min and absorption half-life of 12 min), absorption is essentially complete within five absorption half-lives, i.e., 60 min. Therefore, a comparison of AUC data from 0 to 120 min seems to be appropriate. The absolute nasal bioavailability was also calculated for each nasal preparation by comparing its AUC to that following intravenous injection of 0.25 U/kg insulin.

The percentage decrement in plasma glucose level during 0–120 min as compared to control (intranasal insulin without adjuvants) was calculated using the following equation:

$\%\ D$ = percentage decrement in plasma glucose level during 0–120 min as compared to control of

$$\text{the same dose (insulin without adjuvants)} = \frac{[AUC_{control} - AUC_{adjuvant}] \times 100\%}{AUC_{control}}$$

where $AUC_{control}$ is the area under the plasma glucose curve from 0 to 120 min after nasal administration of insulin without adjuvants, and $AUC_{adjuvant}$ is the area under the plasma glucose curve from 0 to 120 min after nasal administration of insulin in the presence of adjuvants.

Statistical comparisons were made by Student's *t* test. Values are expressed as means ± SE.

RESULTS

As observed in Fig. 1, nasal administration of 5 U/kg insulin dissolved in isotonic 0.07 *M* phosphate, pH 7.4, buffer without adjuvants resulted in practically no changes in the plasma insulin levels, with the values fluctuating slightly about the baseline levels. The average plasma endogenous insulin concentration at time 0 (baseline) was found to be 48.40 ± 8.55 μU/ml, which agreed with the value of 61 μU/ml reported by Cambridge Medical Technology (personal communications). This observation is in accordance with the results of Hirai *et al.* (7,8), who found that insulin administered intranasally without adjuvants at pH 7.4 was not absorbed to any significant extent. Therefore, the inclusion of absorption enhancers is necessary in the nasal formulations.

When insulin was intranasally administered at a dose level of 5 U/kg in the presence of 15 m*M* NaGC, the absorption was considerably enhanced. As seen from Fig. 1, insulin was rapidly absorbed into systemic circulation, reaching a maximum plasma level of 1496 ± 103.4 μU/ml at 15 min, the first time point taken. On the other hand, nasal administration of the same dose of insulin in an emulsion of 5 m*M* linoleic acid did not result in any absorption enhancement (Fig. 1). The plasma insulin levels remained very low about the baseline values and did not significantly differ from that of nasal insulin without adjuvants ($P > 0.05$). However, when insulin was administered together with the mixed micelles consisting of 15 m*M* NaGC and 5 m*M* linoleic acid, the absorption was further enhanced, with the peak plasma insulin concentration of 1910 ± 134.2 μU/ml observed at 15 min. After 15 min, the plasma insulin levels following nasal administration of the peptide with NaGC alone or with mixed micelles gradually declined in parallel and remained relatively high, even at 120 min (about 500 μU/ml).

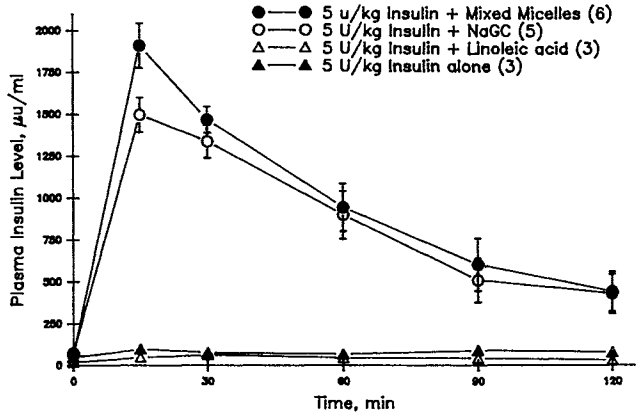

Fig. 1. Plasma insulin concentration–time profiles following intranasal administration of insulin with and without various promoters. Values represent means and SE. Number of rats studied is shown in parentheses.

The percentage increase in the area under the plasma insulin curve during the 120-min period (AUC_{0-120}) produced by mixed micelles over that of NaGC alone was calculated to be 10.5%. Although the mixed micelles appeared to enhance insulin absorption to a greater extent than NaGC, as seen by comparing their plasma insulin AUC_{0-120} values, this is not significant ($P > 0.05$). The absolute bioavailability of intranasal insulin was also calculated for each insulin preparation by comparing the AUC_{0-120} values to that after an intravenous injection. As shown in Table I, the nasal bioavailability, as indicated by the percentage absorbed, was found to be 15.3 and 13.8% when 5 U/kg of insulin was administered with the mixed micelles and NaGC, respectively. Figure 2 represents plasma insulin concentration–time profiles following nasal administration of insulin with the mixed micelles at two different doses, along with data from 0.25 U/kg intravenous administration. The nasal administration of 2 U/kg insulin resulted in an average AUC value which is about 2.24 times lower than that of the higher insulin dose (5 U/kg). The peak plasma level from the 2 U/kg dose also decreased proportionately as compared to the 5 U/kg dose (2.49-fold decrement). The result indicated that absorption of insulin in the presence of mixed micelles probably occurs by passive diffusion across the rat nasal mucosa. Nasal administration of the mixed micelles without insulin, on the contrary, caused no changes in the basal levels of endogenous insulin (Fig. 2), implying that the adjuvants themselves did not interfere with the insulin kinetics or analysis.

Although the AUC_{0-120} values (and thus the nasal bioavailabilities) did not differ significantly between the two types of adjuvants, the peak plasma level of intranasal insulin with mixed micelles was significantly greater than that of insulin with NaGC (1910 vs 1496 μU/ml; $0.01 < P < 0.05$). The marked difference in C_{max} (about 400 μU/ml) implied that the mixed micelles were highly effective in rapidly delivering insulin across the nasal mucosa into systemic circulation.

To prove that the nasally absorbed insulin was active biologically, measurements of the plasma glucose levels following intranasal administration of insulin in the presence of NaGC and mixed micelles were made and compared to the control nasal insulin without adjuvants. As illustrated in Fig. 3, both adjuvants significantly produced hypoglycemic effect by lowering plasma glucose levels from the initial values ($P < 0.0001$). On the other hand, there was practically no decrease in plasma glucose levels following intranasal administration of insulin in the absence of adjuvants. Plasma glucose remained relatively unchanged, about the initial level throughout the 120-min period. This was in agreement with the plasma insulin data (Fig. 1), which showed no sign of significant absorption when insulin was administered alone.

The decrease in plasma glucose started as early as 15 min, the first sampling time point, and was superimposable during the first 30 min for both adjuvants. However, after 30 min the glucose level following intranasal insulin with NaGC remained relatively constant at about 40–45% of the initial level. At 60 min the glucose concentration remaining was

Table I. Pharmacokinetic Parameters of Insulin After Intravenous and Intranasal Administration to Rats; Values = Mean ± SE (n = 3–6 Rats)

Route of Admin.	Dose (U/kg)	Adjuvant	C_{max}	$(AUC)_{0-120}$, (μU/ml) · min[a]	% Absorbed[b]
i.v.	0.25	None	1,162.8 ± 287.1	37,582.8 ± 3,413.9	—
i.n.	2	MM[c]	767.5 ± 64.5	51,399.3 ± 16,534.7	17.10
i.n.	5	MM[c]	1,910.0 ± 134.2	115,037.5 ± 11,989.9	15.30
i.n.	5	NaGC[d]	1,496.0 ± 103.4	104,115.0 ± 11,949.5	13.85

[a] $(AUC)_{0-120}$ = area under the plasma insulin concentration vs time curve from 0 to 120 min.
[b] $[(AUC)_{i.n.}/(AUC)_{i.v.}] \times [dose_{i.v.}/dose_{i.n.}] \times 100\%$.
[c] Mixed micelles consisting of 15 mM NaGC and 5 mM linoleic acid.
[d] Concentration of NaGC = 15 mM.

about 40% and appeared to rise slowly over the next hour. On the other hand, plasma glucose after intranasal insulin with mixed micelles continued to drop after 30 min, reaching the minimum at 60 min (about 30% of the initial value), and stayed at this low level for up to 120 min without a sign of increasing back to the normoglycemic state.

The percentage total decrement in plasma glucose level (% D) during 0–120 min, as compared to nasal insulin without adjuvants (control), was then determined for each type of the two promoters. The values are reported in Table II. As seen from this table, mixed micelles containing 15 mM NaGC and 5 mM linoleic acid produced a total decrease in plasma glucose of about 55%, whereas NaGC alone resulted in only a 47% decrease. In addition, the difference between the two values was significant ($0.01 < P < 0.05$). Therefore, the mixed micelles appeared to be more effective than NaGC alone in eliciting hypoglycemic response. The *in vivo* absorption data seemed to substantiate the previous findings on [D-Arg^2]kyotorphin that the mixed micelles had a greater adjuvant activity than the bile salt or fatty acid alone in promoting nasal absorption of the dipeptide (1). As observed from Fig. 1, an emulsion of 5 mM linoleic acid was not effective at all in enhancing insulin nasal absorption.

The practical use of absorption promoters depends not only on their effectiveness, but also on their nondestructive cellular mechanisms of promotion. It is possible that the enhancement in absorption could be attributed to damage of the nasal epithelium induced by the adjuvants. Therefore, a short-term histological study was conducted to investigate if the mixed micelles can cause marked or severe morphological changes in the rat nasal mucosa. The control groups, either untreated or treated with buffer only, showed no sign of lesions. The epithelial cells remained unaffected in all sections studied (Fig. 4A).

However, all the other groups exposed to adjuvants manifested certain degrees of morphological changes. Most of the changes were found in levels 3 and 4 of the ethmoid recess. This might be a result of the positioning of the rat during the experiment. Each rat was lying on its back and the head was slightly lifted to prevent drainage of the administered solution from the nares. The solution thus appeared to accumulate in the posterior portion of the nasal cavity (sections or levels 3 and 4). The first section was unaffected in all

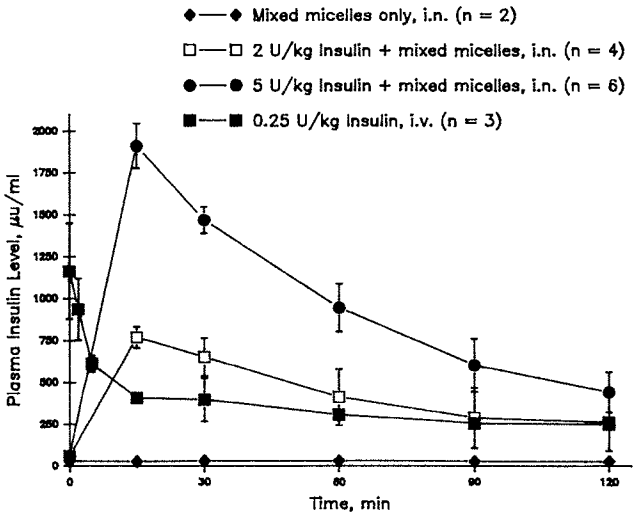

Fig. 2. Plasma insulin concentration–time profiles following intravenous and intranasal administrations of insulin at various doses. Values represent means and SE. Number of rats studied is shown in parentheses.

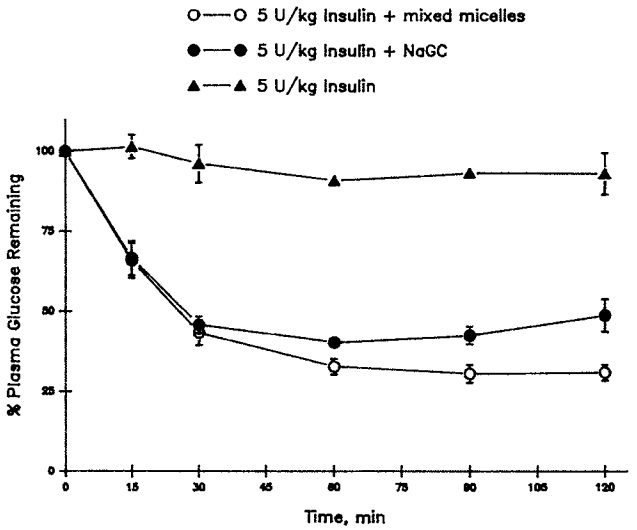

Fig. 3. Hypoglycemic response following intranasal administration of insulin with and without adjuvants. Each value is mean and SE (n = 3–6 rats).

Table II. Comparison of Percentage Decrement in Plasma Glucose Level Following Nasal Administration of Insulin in the Presence of Different Adjuvants; Values = Mean ± SE (n = 3–6 Rats)

Route of Admin.	Dose (U/kg)	Adjuvant	$(AUC)_{0-120}$, % · min[a]	% D[b]
i.n.	5	None	11,344.3 ± 371.3	Control
i.n.	5	NaGC[c]	5,070.5 ± 271.3	55.30 ± 2.39
i.n.	5	MM[d]	5,993.3 ± 168.5	47.17 ± 1.49

[a] $(AUC)_{0-120}$ = area under the plasma glucose concentration vs time curve from 0 to 120 min.
[b] Percentage decrement in plasma glucose level during 0–120 min as compared to control (5 U/kg insulin without adjuvants).
[c] Concentration of NaGC = 15 mM.
[d] Mixed micelles consisting of 15 mM NaGC and 5 mM linoleic acid.

treatments, with some changes observed in level 2. In addition, the lesions were unilateral, i.e., occurred in only one side of the nasal cavity. This was due to the fact that the solution was administered to only one nostril in all experiments. Therefore, the other side of the cavity which was separated by the nasal septum was totally unaffected and could serve as its own control. This observation indicated the high degree of localization of the adjuvants inside the nasal cavity.

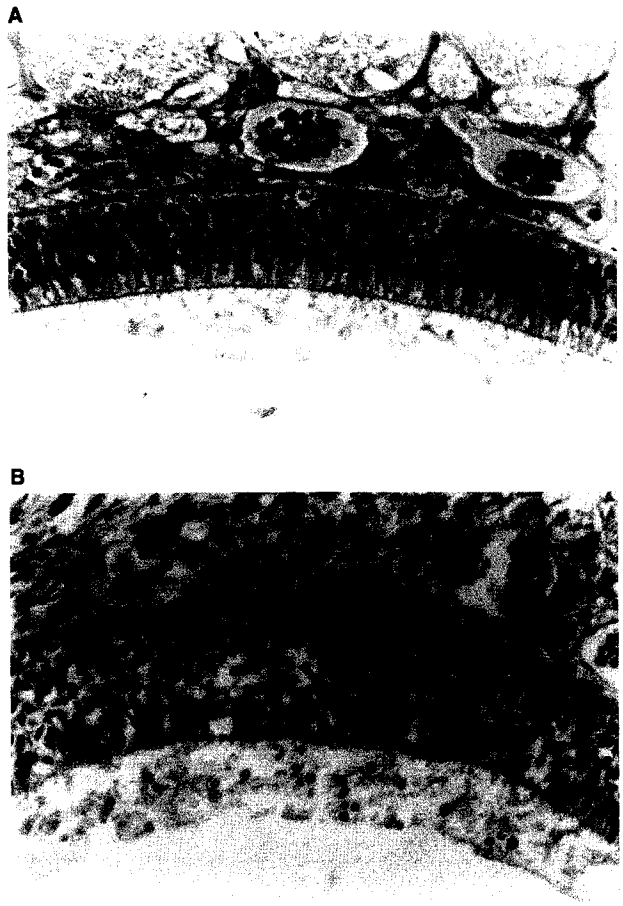

Fig. 4. Typical light micrographs of the nasal mucosa of the untreated control rat (A) and after 1 hr of exposure to mixed micelles (B). × 25; reduced 50% for reproduction.

Figure 4B is typical of the light micrograph of the rat nasal epithelium following exposure to the mixed micelles for 1 hr. The observed changes were characterized by minimal to mild hydropic degeneration and mild nuclear pycnosis in olfactory epithelium of the dorsal meatus and ectoturbinates. The effects were slightly intensified after a longer exposure period of 3 hr. The results after 5 hr of exposure to the mixed micelles were not distinguishable from those after 3 hr, with overall mild to moderate severity. Hydropic degeneration is characterized as swelling and vacuolation of the epithelial cells due to accumulation of fluid such as water. Pycnotic nuclei, on the other hand, are identified as contracted, dark-staining nuclei which are indicative of mucosal damage. However, the extent of such damage was only mild to moderate and was similar with or without added insulin. No marked or severe alterations such as erosion or loss of epithelial surface were observed in any case, indicating that the epithelium remained intact.

DISCUSSION

Although the mixed micelles produced a greater plasma insulin concentration than did NaGC, this was significant only at 15 min, whereas the difference in concentration at other time points was not (Fig. 1). Two possible explanations are available for the only slight increment in the AUC of plasma insulin when the mixed micelles were used instead of NaGC. First, the loss of insulin from the blood does not necessarily indicate that insulin is no longer bioavailable. Rapid distribution of insulin between the plasma and the extravascular pool (including peripheral tissues and receptors) is well documented (14). The possible differences in the distribution rate may partly explain the significantly greater hypoglycemic response observed with mixed micelles than with NaGC despite the similarity in plasma insulin profiles between the two types of adjuvants (Fig. 3). Second, NaGC alone or mixed micelles may promote insulin nasal absorption via both transcellular and paracellular pathway. Evidence for the transcellular transport has been detected in the case of [D-Arg^2]kyotorphin (1). However, as questioned by Lee *et al.* (15), the fate of the intracellular insulin is not clear. Therefore, it is possible that insulin entering the cytoplasm of the nasal epithelial cells might be hydrolyzed, and only insulin localized in the paracellular space is free to diffuse into the systemic circulation. This could result in the only slight enhancement in nasal absorption over the bile salt alone because mixed micelles promote transcellular transport to a greater extent than bile salt alone (1). To prove this hypothesis, however, more experiments are needed such as immunohistological studies to determine the localization of insulin in the nasal mucosa following its administration with mixed micelles (15).

In Fig. 3, the concentration of the plasma glucose following insulin administration had been normalized to the percentage of the initial values. It is well known that surgical stress can cause an overall increase in the plasma glucose concentration due to stimulation of the sympathetic nervous system (2,16). However, this effect varies from rat to rat and may lead to a variability in the initial glucose values. Therefore, the normalization of plasma glucose levels with respect to the initial state appeared to be appropriate in this study.

Results from the histopathologic evaluation indicated that mixed micelles between NaGC and linoleic acid induced some morphological changes such as necrosis in the nasal mucosa. The effect, however, was slight and highly confined. The epithelium remained intact in all treatments.

It is interesting to note that when the mucosal damage by the mixed micelles was compared to that of NaGC with the same exposure period (3 hr), NaGC appeared to produce slightly less hydropy. Only minimal hydropic degeneration and mild pycnotic nuclei were observed. In one rat, the changes could not be detected and the result was not different from the control treatment. Nevertheless, this was not unexpected. Since the fatty acid component of the mixed micelles has been shown to play a critical role in enhancing mucosal permeability (1,17,18), its direct effect on the epithelium is possible. However, based on the results observed here, the suggestion made by Feldman and Gibaldi (19) that lipids may have a protective effect against the intestinal membrane damage caused by bile salt may not be applicable to the nasal mucosa. Nonetheless, mixed micelles between NaGC and linoleic acid were far less irritative than deoxycholate, a more hydrophobic dihydroxy bile salt. Deoxycholate was found to cause a complete loss of epithelial surface even at 0.5% concentration (10).

Considering the 5-hr exposure time, mixed micelles appeared to have mild to moderate effects on the integrity of the nasal mucosa. In this study, the esophagus was cannulated in all rats, thus preventing the loss of the solution into the GI tract. However, during actual administration, the drug and adjuvant will be removed from the nose within a short period via a mucociliary clearance system of the nasal epithelium. Therefore, the safety and effectiveness of the adjuvants will rely partly on the rapid delivery of the drug molecules into systemic circulation and on the rapid clearance of the potentially irritating adjuvants from the mucosal site. Rapid nasal absorption of insulin by mixed micelles has already been demonstrated. However, more histological studies are necessary to include subchronic and chronic nasal administration before a definite conclusion regarding the feasibility of the mixed micelles can be established for clinical use.

In conclusion, the results obtained from this study substantiated previous findings that mixed micelles consisting of NaGC and linoleic acid were effective in increasing the amount of insulin absorbed into the blood. The promoting effect was also superior to the bile salt or fatty acid alone, as observed from the hypoglycemic response and peak plasma insulin level. Furthermore, the absorption was very rapid, with the peak plasma insulin concentration observed at the first time point taken (15 min). Plasma glucose concentration decreased by as much as 70% from the initial value and remained at this level for up to at least 2 hr. The nasal bioavailability of insulin with mixed micelles was about 15–17%, which was comparable to that reported for nasal insulin in the presence of other novel adjuvants such as sodium dihydrotaurofusidate (20).

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REFERENCES

1. P. Tengamnuay and A. K. Mitra. *Pharm. Res* 7:127–133 (1990).
2. B. J. Aungst, N. J. Rogers, and E. Shefter. *J. Pharmacol. Exp. Ther.* 244:23–27 (1988).
3. G. S. Gordon, A. C. Moses, R. D. Silver, J. S. Flier, and M. C. Carey. *Proc. Natl. Acad. Sci.* 82:7419–7423 (1985).
4. A. C. Moses, G. S. Gordon, M. C. Carey, and J. S. Flier. *Diabetes* 32:1040–1047 (1983).
5. A. E. Pontiroli, M. Alberetto, A. Calderara, E. Pajetta, and G. Pozza. In S. S. Davis, L. Illum, and E. Tomlinson (eds.), *Delivery Systems for Peptide Drugs,* Plenum Press, New York, 1986, pp. 243–248.
6. S. Hirai, T. Ikenaga, and T. Matsuzawa. *Diabetes* 27:296–299 (1978).
7. S. Hirai, Y. Takatsuka, and H. Mima. *Int. J. Pharm.* 9:165–172 (1981).
8. S. Hirai, Y. Takatsuka, and H. Mima. *Int. J. Pharm.* 9:173–184 (1981).
9. G. S. M. J. E. Duchateau, J. Zuidema, and F. W. H. M. Merkus. *Int. J. Pharm.* 31:193–199 (1986).
10. S. J. Hersey and R. T. Jackson. *J. Pharm. Sci.* 76:876–879 (1987).
11. A. L. Daugherty, H. D. Liggitt, J. G. McCabe, J. A. Moore, and J. S. Patton. *Int. J. Pharm.* 45:197–206 (1988).
12. S. Hirai, Y. Takatsuka, T. Matsuzawa, and H. Mima. *Int. J. Pharm.* 7:317–325 (1981).
13. J. T. Young. *Fund. Appl. Toxicol.* 1:309–312 (1981).
14. J. Radziuk and T. Morishima. *Adv. Exp. Med. Biol.* 189:247–276 (1985).
15. W. A. Lee, R. A. Ennis, and L. C. Foster. *Proc. Int. Symp. Control. Rel. Bioact. Mater.* 15:70–71 (1988).
16. L. L. Bellinger and V. E. Mendel. *Proc. Soc. Exp. Biol. Med.* 148:5–8 (1975).
17. N. Muranushi, M. Kinugawa, Y. Nakajima, S. Muranishi, and H. Sezaki. *Int. J. Pharm.* 4:271–279 (1980).
18. N. Muranushi, Y. Nakajima, M. Kinugawa, S. Muranishi, and H. Sezaki. *Int. J. Pharm.* 4:281–290 (1980).
19. S. Feldman and M. Gibaldi. *Proc. Soc. Exp. Biol. Med.* 132:1031–1033 (1969).
20. J. P. Longenecker. In S. S. Davis, L. Illum, and E. Tomlinson (eds.), *Delivery Systems for Peptide Drugs,* Plenum Press, New York, 1986, pp. 211–220.