Pharmaceutical Research, Vol. 7, No. 2, 1990

Research Article

Bile Salt–Fatty Acid Mixed Micelles as Nasal Absorption Promoters of Peptides. I. Effects of Ionic Strength, Adjuvant Composition, and Lipid Structure on the Nasal Absorption of [D-Arg^2]Kyotorphin

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Received November 28, 1988, accepted August 15, 1989

Bile salts and synthetic surfactants have been used to promote nasal absorption of peptide drugs. Although a marked increase in nasal absorption has been achieved, this may not be adequate and the possibility of adjuvant-induced membrane toxicity exists. The present study employs a rat *in situ* nasal perfusion technique and mixed micelles between sodium glycocholate (NaGC) and various lipids as potential nasal absorption enhancers of a stable model dipeptide, [D-Arg^2]kyotorphin. NaGC alone enhanced the nasal absorption of the dipeptide in a concentration-dependent manner. When linoleic acid was added to form mixed micelles with NaGC, the absorption was further enhanced ($P < 0.01$). The effect of mixed micelles was synergistic and much greater than with single adjuvants. Increasing ionic strength was found to increase the adjuvant activity of both NaGC and NaGC–lipid mixed micelles. Structure of the lipid component of the mixed micelles also affected the adjuvant potency. Oleic acid, a *cis*-unsaturated fatty acid, was more effective than elaidic acid, the *trans*-isomer, whereas *cis*-linoleic acid and *trans*-linolelaidic acid were equally effective ($\alpha = 0.05$). Mixed micelles of monoglycerides such as monoolein and monolinolein were also more effective than NaGC alone ($\alpha = 0.05$). Micellar solubilization of these polar lipids by NaGC appears to be important for nasal absorption enhancement to occur. Reversal of the membrane permeability was also observed within approximately 20–40 min after removal of the adjuvants from the rat nasal cavity. These observations are similar to the effects of mixed micelles on the rectal mucosa and may involve the same mechanism.

KEY WORDS: mixed micelles; bile salts; sodium glycocholate; unsaturated fatty acids; nasal absorption enhancers; [D-Arg^2]kyotorphin; ionic strength; adjuvant composition; lipid structure.

INTRODUCTION

Nasal administration appears to offer a promising alternative to the delivery of compounds that are poorly absorbed from the gastrointestinal tract and/or subject to extensive hepatic first-pass metabolism. The range of compounds investigated for possible nasal administration varies greatly from very lipophilic drugs to polar, hydrophilic molecules including peptides and proteins. In general, low molecular weight lipophilic drugs such as steroidal hormones and β-blockers appear to be absorbed well across the nasal mucosa with relatively high nasal bioavailability (1–3).

Greater challenges have been encountered when hydrophilic compounds were administered intranasally. Nasal absorption of peptides and proteins, in particular, has been less successful and often requires certain types of adjuvants to effect absorption to any significant extent (4–7). Recently it has been suggested that the nasal mucosa is only moderately permeable to water-soluble drugs (8) and has a molecular weight cutoff point of about 1000 (9). Above this value, the nasal bioavailability decreases considerably and the use of adjuvants may be necessary.

Among the adjuvants used in the nasal formulations of peptides, bile salts and other surfactant-type adjuvants are most common. Nasal absorption of insulin, for example, was extensively investigated using different types of surfactants (6,7,10). Despite the significant improvement in the hypoglycemic effects, the nasal bioavailability was found to be only about 10 to 30% of the intravenous administration. In addition, several toxic effects and histological damage have been reported from the use of these adjuvants (8,11).

Recently the use of several adjuvant combinations has received greater attention. Muranishi and other workers have reported the successful use of lipid–surfactant mixed micelles to promote the intestinal absorption of several poorly absorbed drugs such as heparin, aminoglycosides, and peptides (12–14), which resulted in a much greater extent of absorption enhancement and possibly less deleterious effects on the mucosa than the bile salts alone. However, most of the work has been limited to improvement of intes-

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tinal absorption, especially the large intestine. No attempts of using coadjuvants have been reported with respect to nasal drug absorption enhancement (15). It is likely that the nasal mucosa might be affected in the same manner. This report describes the use of bile salt–unsaturated fatty acid mixed micelles as potential nasal absorption enhancers of peptides. L-Tyr-D-Arg or [D-Arg^2]kyotorphin, a biologically active dipeptide, was chosen as a model peptide in this study due to its stability in the rat nasal cavity as well as its poor membrane permeability. Physicochemical parameters such as the effects of ionic strength, adjuvant composition, and lipid structure on the nasal absorption of this dipeptide were investigated.

MATERIALS AND METHODS

Materials. Kyotorphin (L-tyrosyl-L-arginine) and its D analogue (L-tyrosyl-D-arginine or [D-Arg^2]kyotorphin) were obtained through Sigma Chemical Co., St. Louis, MO. All the adjuvants were at least 99% pure and were also purchased from Sigma. These are sodium glycocholate (NaGC), oleic acid, elaidic acid, linoleic acid, linolelaidic acid, linolenic acid, 1-monoolein, 1-monolinolein, and methyl linoleate. Other reagents were also of analytical grade. All the solutions were freshly prepared and filtered prior to use.

In situ *Perfusion of the Rat Nasal Cavity.* The technique used in this investigation involved *in situ* perfusion of the rat nasal cavity. This method was developed by Hirai (16) and has been demonstrated by Huang to be well correlated with the *in vivo* blood data (17). Details of the experiment have been described by these authors. In our previous report we have successfully characterized the nasal absorption mechanisms of two aromatic amino acids, phenylalanine and tyrosine, in rats using a similar technique (18). Furthermore, this method allows for the detection of any peptide hydrolytic products in the perfusion medium. Therefore, *in situ* nasal perfusion appears to be an appropriate method to assess the effects of various adjuvants on nasal absorption of peptides and to elucidate the mechanisms of absorption enhancement.

Preparation of Mixed Micelles. Each fatty acid was added to a buffer solution containing a mixture of bile salt (NaGC) and [D-Arg^2]kyotorphin, stirred continuously, and finally, sonicated at room temperature for 5 min with a Branson sonicator (Model 3200, Branson Co., Shelton, CT). The solutions were then slowly adjusted to the final volume with buffer solutions such that each solution contained various preselected concentrations of fatty acid and bile salt, whereas the concentration of the dipeptide remained constant at 0.5 m*M*. The appearance of clear mixed micellar solutions was found to depend on the molar ratio of bile salt to lipid as well as on the type of lipids and buffers used. In addition, for comparison purposes, micellar solutions of NaGC alone or suspensions of fatty acid (5 m*M*) in the same buffers were prepared using similar procedures.

Two types of aqueous media having different buffer composition and ionic strengths were employed. These were as follows: buffer I—isotonic 0.07 *M* phosphate buffer, pH 7.4, consisting of 0.014 *M* KH_2PO_4, 0.057 *M* Na_2HPO_4, and 0.070 *M* NaCl (μ = 0.255); and buffer II—isotonic dextrose–0.01 *M* phosphate buffer, pH 7.4, containing 0.004 *M* KH_2PO_4, 0.006 *M* Na_2HPO_4, and 0.256 *M* dextrose (μ = 0.022). Dextrose, a nonelectrolyte, was added to buffer II instead of NaCl in order to adjust the tonicity of the medium without affecting the total ionic strength. The amount of dextrose added was calculated using the NaCl equivalent method. All the solutions and suspensions were used immediately after preparation. Previous experiments using HPLC have shown that the dipeptide was stable in the mixed micelles for at least 24 hr. The net volume of the perfusates was found to be fairly constant (5 ± 0.2 ml) throughout the 1-hr perfusion period.

Analytical Procedure. The concentration of dipeptides remaining in the nasal perfusates was quantitated by reversed-phase HPLC using a solvent delivery pump (model Rabbit HP, Rainin Instrument, Woburn, MA) equipped with a variable wavelength UV detector set at 274 nm. The column consisted of a nonpolar stationary phase (Microsorb C18, 5-μm particle size, Rainin Instrument). Acetonitrile (4.75%, v/v) in 0.01 *M* ammonium formate (pH 4.0) was used as the mobile phase at a flow rate of 1.0 ml/min. The peak heights or areas were calculated with an HP 3390A integrator (Hewlett–Packard, Avondale, PA). An aliquot (25 μl each) of the perfusate was periodically removed from the perfusion setup's reservoir and immediately mixed with the internal standard solution (L-phenylalanine) prior to injection onto the column. The retention volumes for L-tyrosine, kyotorphin, L-phenylalanine, and [D-Arg^2]kyotorphin were 4.1, 6.3, 7.9, and 8.5 ml, respectively. Arginine (both D and L), NaGC and lipids did not interfere with the analysis.

Data Analysis. Since the loss of peptide in the presence of adjuvants was linear, at least during the 60-min perfusion period ($r > 0.90$), the extent of absorption was determined from linear regression of the plots of the percentage dipeptide remaining in the perfusate versus time. The statistical analysis of the data was made by Student's *t* test, analysis of variance, and range test where appropriate. Results are expressed as means ± SE.

RESULTS

In Situ Nasal Perfusion of Kyotorphin and [D-Arg^2]Kyotorphin Without Adjuvants

Kyotorphin (L-Tyr-L-Arg) is a bioactive dipeptide that can stimulate the release of endogenous met-enkephalin (19). [D-Arg^2]Kyotorphin (L-Tyr-D-Arg) is a D analogue possessing a greater analgesic effect than the L isomer due to its ability to resist degradation by peptidases (20). As depicted in Fig. 1, when the two dipeptides were perfused through the rat nasal cavity, kyotorphin was found to be enzymatically cleaved, resulting in the rapid loss of the dipeptide from the perfusate with a concomitant increase in the concentration of L-tyrosine degradation product. [D-Arg^2]Kyotorphin, on the other hand, was quite stable in the nasal perfusate and L-Tyr did not appear even after 90 min of perfusion.

However, even in the absence of enzymatic degradation, it is evident from Fig. 1 that [D-Arg^2]kyotorphin was hardly absorbed from the perfusate. Its concentration in the perfusate was found to remain fairly constant during the entire perfusion period. Hence, this polar molecule, although small in size, may not be readily transported across the rat

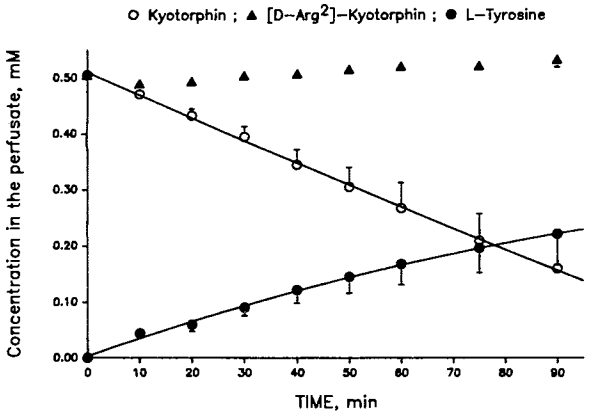

Fig. 1. Stability of kyotorphin and [D-Arg^2]kyotorphin during nasal perfusion. The dipeptides were dissolved in buffer I without any adjuvants. Kyotorphin was rapidly degraded during the perfusion, resulting in the appearance of L-tyrosine. [D-Arg^2]Kyotorphin, on the other hand, was very stable in the perfusate. Each value is the mean ± SE (n = 3 rats).

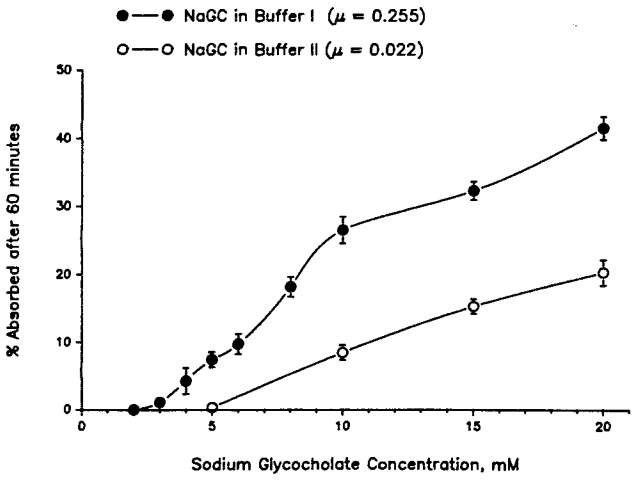

Fig. 2. Effect of NaGC on nasal absorption of [D-Arg^2]kyotorphin. Perfusion was carried out at 37°C in both buffer I and buffer II. Each value is the mean ± SE (n = 4–6 rats).

nasal mucosa. Varying the initial perfusate concentration of this dipeptide also did not show any absorption (or degradation) from the perfusate, as opposed to amino acids such as L-phenylalanine (18). L-Phenylalanine has been found to be actively absorbed in a concentration-dependent manner and the loss of the amino acid from the perfusate was very rapid despite its high polarity. Thus, compared to amino acids, [D-Arg^2]kyotorphin may not have an effective carrier for the nasal transport. Due to its intrinsically low nasal permeability, this compound appeared to be an appropriate model dipeptide to study the adjuvant effects. Its biological significance, low molecular weight, and ability to resist enzymatic hydrolysis also contributed to the selection of this dipeptide.

Effects of Sodium Glycocholate and Ionic Strength on the Nasal Absorption of [D-Arg^2]Kyotorphin

The effects of adjuvants were studied first by including a bile salt into the perfusion medium containing the dipeptide. Since [D-Arg^2]kyotorphin was found to be very stable in the nasal perfusate regardless of the presence of the adjuvants employed in this study, any loss of the dipeptide from the perfusate should be indicative of its absorption across the nasal mucosa. In addition, since no change in the perfusate concentration of [D-Arg^2]kyotorphin was observed when the dipeptide alone was perfused, the interactions of the dipeptide with the tubing as well as with the surface mucus layer of the nasal mucosa were considered negligible.

The conjugated trihydroxy bile salt NaGC was used here as a model bile salt due to its relatively low toxicity as compared to synthetic surfactants and dihydroxy bile salts (21,22). Initially, solutions containing 0.5 mM [D-Arg^2]-kyotorphin and various concentrations of NaGC (0–20 mM) in both buffer I and buffer II were perfused through the rat nasal cavity for 60 min. The extent of nasal absorption was then determined by analyzing the concentration of the remaining dipeptide in the perfusate and plotting it as a function of the bile salt concentration. Figure 2 represents such a plot. It is clear from this figure that the absorption increased as the concentration of NaGC was increased in both perfusion media. More interestingly, the absorption of [D-Arg^2]-kyotorphin was observed to be much greater ($P < 0.01$) from buffer I (higher ionic strength medium) than from buffer II over all the NaGC concentrations studied.

Effects of Bile Salt–Lipid Mixed Micelles

Effects of Ionic Strength and Varying Sodium Glycocholate Concentration

The effects of NaGC–fatty acid mixed micelles on the nasal absorption of [D-Arg^2]kyotorphin were then investigated to examine if there was any further enhancement of absorption. Figure 3a represents a plot of the percentage [D-Arg^2]kyotorphin absorbed after 60 min of nasal perfusion of the dipeptide in the presence of NaGC–linoleic acid mixed micelles contained in buffer I. The addition of only 5 mM linoleic acid into NaGC solutions significantly enhanced the absorption even further over the entire concentration range of NaGC studied ($P < 0.01$).

The promoting effect of the mixed micelles appeared to be synergistic, i.e., greater than that produced by bile salt or fatty acid alone or a combination thereof, especially in the NaGC concentration range of 2–8 mM (Fig. 3a). For example, 5 mM NaGC alone enhanced the nasal absorption of the dipeptide to only 7.4%, whereas the addition of 5 mM linoleic acid to form mixed micelles caused an increase in the absorption to 26.2% (Fig. 3a). Furthermore, a 2 mM NaGC solution did not produce any observable absorption but the coexistence of linoleic acid still enhanced the absorption to about 16.8% despite the fact that a clear mixed micellar solution was not obtained due to a larger proportion of fatty acid (5 mM) over the bile salt (2 mM). A clear mixed micellar solution was obtained in all other cases where the molar ratio of NaGC to linoleic acid in this buffer was $\geq$1. Maximum absorption (about 46–48%; Fig. 3a) was observed between 15:5 and 20:5 mM ratios of NaGC to linoleic acid. A suspension of 5 mM linoleic acid also slightly promoted absorption.

Figure 3b, which was obtained from the experiments performed in a low-ionic strength medium (buffer II), also

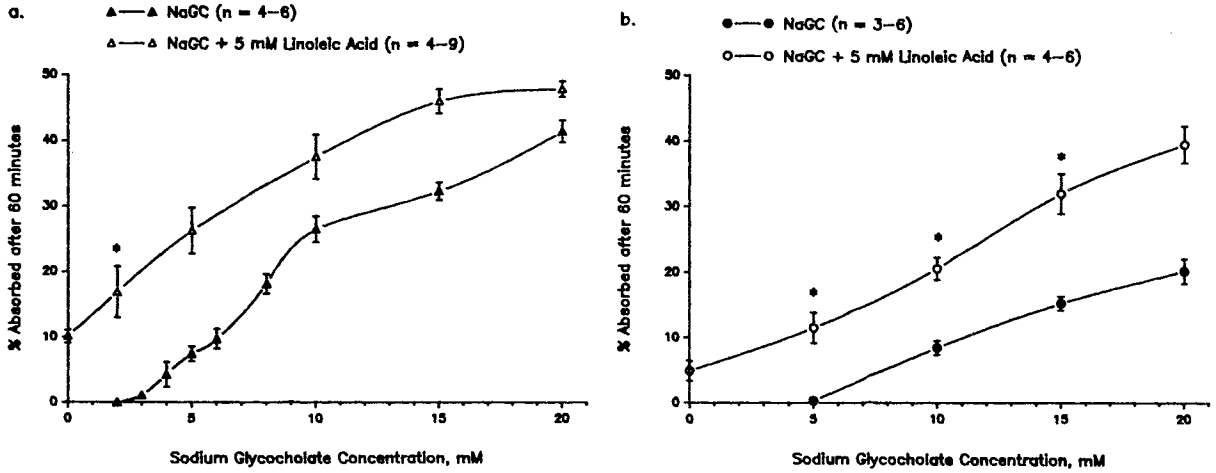

Fig. 3. Effect of NaGC–linoleic acid mixed micelles relative to NaGC alone on nasal absorption of [D-Arg^2]kyotorphin. Perfusion was carried out at 37°C in buffer I (a) and buffer II (b). Each value is the mean ± SE (n = 3–9 rats). An asterisk indicates an unclear mixed micelle preparation.

demonstrated greater effectiveness of the mixed micelles ($P < 0.01$), although their enhancing effects appeared to be somewhat parallel over the entire bile salt concentration range. Furthermore, it was noticed that a clear mixed micellar solution was obtained only at the highest ratio of NaGC to linoleic acid (20:5 mM). At lower ratios of bilt salt to fatty acid, the preparations had an emulsion-like appearance (somewhat opaque). However, the solutions became clearer as the concentration of NaGC was increased until completely clear mixed micelles were formed at a 20:5 mM ratio.

When Fig. 3a was compared to Fig. 3b, the influence of ionic strength was evident. At the same molar ratio of bile salt to fatty acid, the percentage absorption caused by the adjuvants was always greater in buffer I to which extra NaCl had been added than in buffer II ($P < 0.01$). However, once visually clear mixed micelles were formed in buffer II, the difference in adjuvant activity of the two media, although still significant, was much smaller than at other ratios.

Effects of Varying Linoleic Acid Concentration

Since dipeptide absorption induced by the mixed micelles seemed to increase linearly as a function of NaGC concentration and level off at a bile salt concentration of about 15 mM (Fig. 3a), all subsequent studies were performed by maintaining the NaGC concentration at 15 mM using buffer I as the medium. Figure 4 (upper curve) depicts a plot of percentage dipeptide absorbed versus concentration of linoleic acid in the presence of 15 mM NaGC. When linoleic acid concentration was increased from 5 to 15 mM, there was no significant difference in the extent of absorption induced by the mixed micelles (one-way ANOVA, $p >$ 0.05). Similar results were obtained at a NaGC concentration of 10 mM (Fig. 4, lower curve). Moreover, when the linoleic acid concentration was twice that of NaGC, the preparation became unclear, as the excess lipid could not be solubilized in the bile salt micelles. The absorption also decreased to the same level as that produced by NaGC alone.

Effects of Fatty Acid Unsaturation and Double Bond Configuration

Since Fig. 4 revealed that a 15:5 mM ratio of NaGC to linoleic acid was optimum, this ratio was used in subsequent studies to minimize any possible toxicity associated with the adjuvant concentration. Five unsaturated fatty acids were employed, each having varying degrees of unsaturation and different double bond configurations (*cis* and *trans*). All have 18 carbon atoms in the molecule. These are elaidic acid (*trans*-9-octadecenoic acid), oleic acid (*cis*-9-octadecenoic acid), linolelaidic acid (*trans*-9,*trans*-12-octadecadienoic acid), linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid), and linolenic acid (*cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid).

When nasal absorption of the dipeptide was compared between the micellar solution of NaGC (15 mM) and the mixed micelles of each fatty acid, there was a significant difference in the extent of absorption among the six groups (Fig. 5, one-way ANOVA, $P = 0.0001$). A modified Tukey's test was then applied to make multiple comparisons between

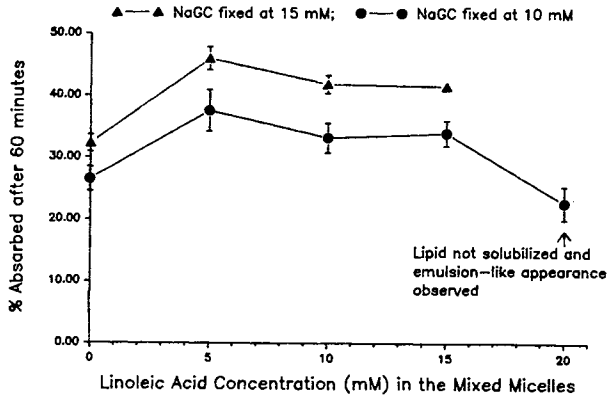

Fig. 4. Effect of varying linoleic acid concentration in the mixed micelles on the nasal absorption of [D-Arg^2]kyotorphin. NaGC in the mixed micelles was fixed at 15 mM (upper curve) and 10 mM (lower curve), respectively. Medium was buffer I. Each value is the mean ± SE (n = 3–9 rats).

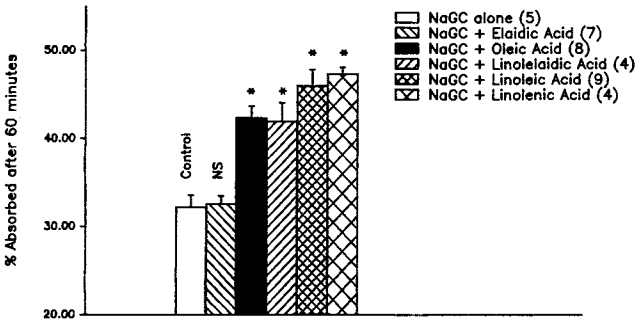

Fig. 5. Effects of degree of unsaturation and double bond configuration (*cis* and *trans*) of fatty acids in the mixed micelles on nasal absorption of [D-Arg^2]kyotorphin. The ratio of NaGC to lipid was 15 to 5 m*M* in buffer I. There was a significant difference in adjuvant potency among all six groups (one-way ANOVA, $P = 0.0001$). (*) Significantly more effective than control (NaGC alone) after applying modified Tukey's test at $\alpha = 0.05$. (NS) Not significantly different from control. Each value is the mean ± SE. Number in parentheses represents number of animals used.

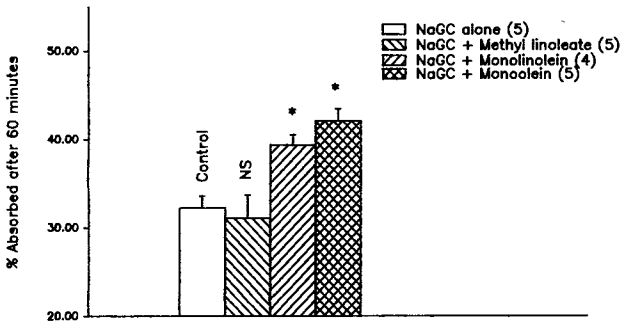

Fig. 6. Effects of methyl ester and monoglycerides in the mixed micelles on nasal absorption of [D-Arg^2]kyotorphin. The ratio of NaGC to lipid was 15 to 5 m*M* in buffer I. There was a significant difference in adjuvant potency among all four groups (one-way ANOVA, $P = 0.0007$). (*) Significantly more effective than control (NaGC alone) after applying modified Tukey's test at $\alpha = 0.05$. (NS) Not significantly different from control. Each value is the mean ± SE. Number in parentheses represents number of animals used.

these groups. It was found that all the fatty acid mixed micelles tested, except elaidic acid, were significantly more effective than NaGC alone ($a = 0.05$). The adjuvant potency of elaidic acid mixed micelles, on the other hand, was not significantly different from that of NaGC micellar solutions and, consequently, was lower than the other fatty acids. In addition, there was no significant difference in the adjuvant potency among oleic acid, linolelaidic acid, linoleic acid, and linolenic acid mixed micelles.

Effects of Fatty Acid Ester and Glyceride

Mixed micelles of NaGC with methyl linoleate, monolinolein, or monoolein at a ratio of 15 to 5 m*M* were studied by the nasal perfusion method.

To compare the adjuvant activity of these lipid mixed micelles relative to NaGC solution (control), ANOVA was first applied in a manner similar to the previous testing on the double bond effect. As expected, a significant difference in the extent of nasal absorption was found among the four groups (Fig. 6; one-way ANOVA, $P = 0.0007$). A modified Tukey's test was subsequently applied for multiple comparisons of the four adjuvants at $\alpha = 0.05$. The statistical analysis showed that mixed micelles of monoolein and monolinolein significantly promoted absorption of [D-Arg^2]-kyotorphin over the NaGC micellar solution, while mixed micelles containing methyl linoleate had a much lower adjuvant activity and did not result in any enhancing effect over NaGC alone (Fig. 6). In addition, both monoolein and monolinolein mixed micelles demonstrated a similar adjuvant potency.

Reversibility of Nasal Membrane Permeability

As a rough estimate of the damaging effects on the nasal mucosa caused by mixed micelles, the rat nasal cavity was flushed with normal saline for 20 min after having been perfused with a mixed micellar solution containing 15 m*M* NaGC and 5 m*M* linoleic acid. The perfusion was then restarted using the dipeptide solution without any adjuvants. Figure 7 shows that nasal permeability of the rat gradually decreased to its original impermeable state within 20 min (ranging from 20 to 40 min in three rats), as no more loss of the dipeptide was observed. Considering the time of preexposure to the mixed micelles (60 min), forced perfusion, and high amount of adjuvants (5-ml perfusate volume), the results are in agreement with the previous findings on the rectal mucosa that mixed micelles are relatively safe and have a transient effect on the biomembrane (23). However, histological as well as long-term toxicity studies need to be performed to justify their clinical use in the nasal drug delivery systems.

DISCUSSION

This study has clearly demonstrated that nasal mucosal permeability can be enhanced in the presence of bile salt–fatty acid mixed micelles. The enhancing effect is synergistic and much greater than with single adjuvants. The possible mechanisms of absorption promotion induced by bile salts have been well summarized (11,24) and only those related to the action of mixed micelles are cited here for comparison purposes.

Although the data on the effects of ionic strength are

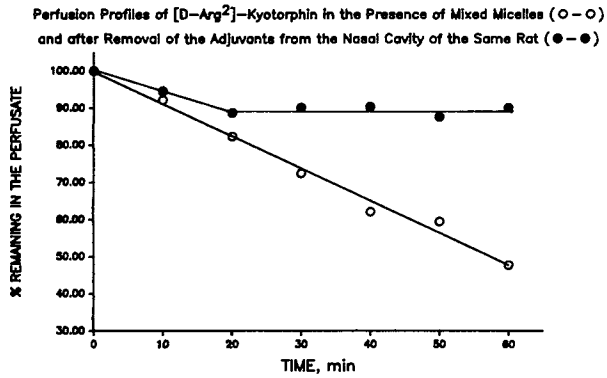

Fig. 7. Reversibility of nasal membrane permeability following removal of the mixed micelles from the rat nasal cavity. Membrane permeability returned to its original impermeable state within 20–40 min after the nasal cavity had been flushed with normal saline.

limited to only two buffers, the trend in the data presented for various systems in Figs. 2, 3, and 4 does appear to support the contention that ionic strength variations affect adjuvant activity of both bile and bile salt–lipid mixed micellar systems and hence influence peptide absorption characteristics. The suggested explanations for this trend may include the fact that increasing ionic strength causes an increase in the surface activity of charged surfactants such as the bile salts, the fatty acids, or their mixed micelles. This increase in surface activity at the higher ionic strength may be sufficient to enhance peptide permeability over that observed for lower ionic strength buffer. Also, it appears unlikely that the absorption of the hydrophilic peptide would be enhanced by solubilization of the peptide in the hydrophobic interior of the micellar aggregates. It is more probable that the monomers are responsible judging from the saturation effects depicted in Figs. 2 and 3.

Another possibility might be an increase in the size and number of NaGC micelles as the counterion concentration [Na^+] is increased, assuming that micellar aggregates are active species in the absorption promotion. Na^+ has been found to increase the micellar weight of NaGC by reducing charge repulsions between NaGC molecules in the micelle (25). Evidence supporting bile salt micelles as active components has been reported by Gordon *et al.*, who observed that deoxycholate was effective in enhancing nasal absorption of insulin only when its concentration was well above the CMC (26)

Shiga *et al.* (27) and Fix *et al.* (28) reported that ouabain had an inhibitory effect on rectal drug absorption promoted by adjuvants such as sodium taurocholate and sodium salicylate. They consequently suggested that active sodium transport may be involved in the mechanism of action of these promoters. Such a phenomenon could lead to a Na^+-dependent influx of water and, as a result, an enhancement in drug absorption via a solvent drag effect. Indeed, Karino *et al.* have demonstrated a high correlation between water influx and intestinal absorption clearance of several drugs (29). In addition, bile salts might have been actively absorbed across the nasal mucosa by flux coupled with Na^+ as observed in the ileum (30). Whether any or all of these phenomena occur in the nasal cavity, however, requires further investigation. The addition of polar lipids as a coadjuvant to form mixed micelles with bile salts can further improve absorption probably via additional mechanisms.

Although the physiology of the nasal cavity is different from that of the intestine, evidence for absorption of fatty acids across the rat nasal mucosa has been reported (31). In the intestine, lipid absorption was found to occur by passive diffusion which is, at least, partially facilitated by micellar solubilization with bile salts (30). Our data from Figs. 3 and 4 clearly show that lipids need to be solubilized in the mixed micelles for significant absorption enhancement to occur. These observations suggest that solubilization of fatty acids by surfactants such as bile salts would allow more lipid molecules to be available at the nasal membrane interface for subsequent absorption and/or interactions with the membrane components, resulting in increased membrane permeability. In this sense, any surfactants, not necessarily bile salts, would be expected to operate. Indeed, Yoshikawa *et al.* (14) found that the use of mixed micelles between nonionic surfactants such as polyoxyethylene castor oil derivative and linoleic acid was also effective in promoting the absorption of interferon from the large intestine. The biological role of NaGC on lipid absorption in the nasal cavity has not been studied and we do not know if bile salts are actively absorbed across the nasal mucosa as in the ileum. However, micellar solubilization of lipids by surfactants reflects a physical phenomenon which should be common for both routes.

Figure 7 demonstrates that the effect of mixed micelles of bile salts with fatty acids on nasal membrane permeability is reversible. The presence of fatty acids or monoolein in the small intestine was also reported to lessen a number of toxic effects associated with bile salts (32). Feldman and Gibaldi observed that the addition of lecithin or monoolein and oleic acid to sodium taurodeoxycholate micelles resulted in a decreased permeability response to salicylate in the everted rat intestine experiment (33). They suggested that incorporation of lipids in the bile salt micelle may reduce the ability of the micelle to solubilize membrane components, which may lead to a protective effect against membrane damage.

Since our results tend to indicate greater adjuvant potency of mixed micelles over bile salts, the promotion mechanism by mixed micelles may not involve membrane solubilization as a major mode of action. The possibility of a transcellular pathway via perturbation of the membrane phospholipid bilayer by lipid component of the mixed micelles has been extensively discussed by Muranushi *et al.* (34,35). Recently, Tomita *et al.* reported that mixed micelles composed of sodium oleate and sodium taurocholate could enhance colonic absorption of cefmetazole and inulin by a paracellular route (36). These adjuvants were found to increase significantly the colonic pore size in the aqueous channel. However, further experimentation is needed to elucidate in detail the promotion mechanisms of mixed micelles in the nasal mucosa.

In conclusion, bile salt–unsaturated fatty acid mixed micelles appear to be effective and probably safe adjuvants in promoting nasal absorption of dipeptide [D-Arg^2]kyotorphin. Bile salts may facilitate transmucosal penetration of fatty acids by solubilizing them in mixed micelles, thus making them more available at the mucosal surface for subsequent absorption. Interactions of these lipids at the level of nasal epithelium may involve both transcellular and paracellular pathways, resulting in the increase in membrane permeability to the otherwise poorly absorbed drugs. Studies are now being conducted to evaluate the effectiveness of the mixed micelles as potential nasal absorption promoters *in vivo*.

ACKNOWLEDGMENTS

This work was supported in part by a Faculty Development Award to A.K.M. by Merck, Sharp and Dohme Research Laboratories. Instrumentation support was provided in part by NIH Biomedical Research Support Grant RR05586 and in part by NIH Grant NS25284.

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