Nasal absorption of insulin: Enhancement by hydrophobic bile salts

(drug absorption/hydrophobicity/mixed micelles/reverse micelles/steroid detergents)

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ABSTRACT We demonstrate that therapeutically useful amounts of insulin are absorbed by the nasal mucosa of human beings when administered as a nasal spray with the common bile salts. By employing a series of bile salts with subtle differences in the number, position, and orientation of their nuclear hydroxyl functions and alterations in side chain conjugation, we show that adjuvant potency for nasal insulin absorption correlates positively with increasing hydrophobicity of the bile salts’ steroid nucleus. As inferred from studies employing various concentrations of unconjugated deoxycholate and a constant dose of insulin, insulin absorption begins at the aqueous critical micellar concentration of the bile salt and becomes maximal when micelle formation is well established. These and other data are consistent with the complementary hypotheses that bile salts act as absorption adjuvants by (i) producing high juxtamelantrum concentrations of insulin monomers via solubilization in mixed bile salt micelles and (ii) forming reverse micelles within nasal membranes, through which insulin monomers can diffuse through paracellular channels from the nares into the blood stream.

Certain small peptides can be absorbed through the nasal mucosa as a “sniff” or directly from aqueous solution (1–5). However, efficacy of absorption is typically low and variable (1–5), and therapeutically important peptides of larger molecular size, such as insulin, are not absorbed to any appreciable degree (6). Within the gastrointestinal tract, bile salts promote the transmembrane movement of endogenous and exogenous lipids (7) and the transmembrane and/or paracellular movement of several small endogenous and exogenous polar molecules—e.g., water (7), inorganic electrolytes (7), polyethylene glycols (8), and oxalate (9). Because of these functions, as well as their detergent-like properties on biomembranes (10), bile salts are potential adjuvants for transmucosal delivery of drugs and have been widely explored for this purpose (11–17). Although there is abundant physical-chemical information concerning the micellar properties of bile salt molecules as well as their interactions with membrane and exogenous lipids (18, 19), little is known about the mechanisms by which these molecules might enhance transmucosal absorption of drugs (17). As shown by us and others, bile salts promote the nasal absorption of insulin in man (20, 21) as well as in laboratory animals (6, 22). Nevertheless, previous studies in rats, employing a range of bile salt species, failed to define any useful structure–function relationships (23). We now report structure–function studies on a series of naturally occurring bile salts by testing their ability to enhance insulin absorption across the human nasal mucosa when administered intranasally as an insulin/bile salt spray. Dramatic differences in insulin absorption were observed between closely related bile salt species; the pattern was shown to be determined by the hydrophilic–hydrophobic balance (24) of the hydroxyl-substituted steroid nucleus and not that of the overall molecule. This correlation suggests strategies for future development of safe and effective insulin-transporting agents.

MATERIALS AND METHODS

Experimental Subjects. We studied 40 healthy human volunteers 19–35 years old who were within 10% of ideal body weight. All subjects gave written informed consent to an experimental protocol approved by the Clinical Investigation Committee of Beth Israel Hospital and were studied in the hospital’s Clinical Research Center. Subjects were studied in the supine position on the morning after an overnight fast. Intravenous catheters were placed in a forearm vein for blood sampling; patency was ensured by the continuous infusion of 0.15 M NaCl at the rate of 15 ml/hr.

Insulin and Bile Salts. Commercially available U-500 regular porcine insulin was obtained from Eli Lilly. One unit (U) of insulin = 42 μg. Bile salts [sodium salts of deoxycholate, glycodeoxycholate, taurodeoxycholate (all 3α,12α-dihydroxy-5β-cholanoates); sodium salts of cholate, glycocholate, and taurocholate (all 3α,7α,12α-trihydroxy-5β-cholanoates)] were purchased from Calbiochem and were purified by recrystallization according to the methods of Pope (25) and Norman (26). Bile acids [ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholanic acid) and chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholanic acid)] were received as generous gifts from Herbert Falk (Falk GmbH, Freiburg, Federal Republic of Germany) and were converted to the sodium salts as described (24). Purity of all bile salts was greater than 98–99% as determined by thin-layer chromatography, reverse-phase high-performance liquid chromatography (HPLC), and titration with HCl (24, 27).

Methods. Immediately prior to use, bile salts were dissolved in 0.15 M NaCl at pH 10 to give concentrations of 2–5% (wt/vol). With 1 M HCl, the pH of each solution was adjusted to 7.4–7.8 except for that of ursodeoxycholate, which was adjusted to 8.1 owing to its insolubility at physiological pH (27). Bile salt solutions were mixed with U-500 regular porcine insulin in 0.15 M NaCl to give final bile salt concentrations of 1% (wt/vol) and sufficient insulin for an intended delivery of 0.5 U/kg of body weight. Since the volume of the administered spray was fixed, and the weight of subjects varied from 40 to 85 kg, the ratio of bile salt to insulin varied over a 2-fold range. Insulin/bile salt solutions were administered within 2 hr of mixing as single sprays in each nostril, employing a metered-pump sprayer (Boehringer Ingelheim, Ridgefield, CT), which delivered 75 ± 8 μl per spray.

Abbreviations: U, unit; emc, critical micellar concentration.
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as absorbed into hydrophilic-hydrophobic salt, for insulin predicted micellar phase composed that the basis of (Altex, capacities monomer: radioimmunoassay Serum insulin Baxter-Travenol, allowed 7420 bile salts to of mM) of regular porcine samples (Clinical MA). The_locator/et intervals

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Fig. 1. Dependence of peak serum insulin on bile salt hydrophobic retention factor. As calculated according to Armstrong and Carey (2), decreases with increasing hydrophobic...bile salts face outwards whereas the hydrophilic sides of the...association (18, 19, 35). To explore whether the...hydrophilic side of the bile salt is important in the...micelles. The_locator/et interval

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The mechanism by which bile salts enhance absorption of insulin (or other drugs) across biomembranes is not known. To be absorbed from the nasal mucosa to reach the blood circulation, insulin molecules must be transported through (or between) a number of membrane barriers in series. These include typical and basal membranes of mucosal cells, the lamina propria, and capillary endothelial cells (36). At physiologic pH, insulin molecules are only sparingly soluble as monomers in aqueous systems (37) and in biomembranes (38) (≈170 pM). Interestingly, such insulin concentrations approximate both basal and stimulated physiological blood levels (39, 40). Solutions of so-called “soluble” insulin in various commercial formulations contain particles that range in size from ≈100 Å to >100,000 Å (41), suggesting that a high proportion of aqueous insulin molecules are present as either microcrystals or polyomers. This self-aggregation process occurs because the exterior of the insulin monomer, as shown crystallographically, has two large nonpolar (hydrophobic) surfaces on opposing sides (42); one side is involved in dimer formation, and the other in higher aggregate (hexamer–polymer) formation (42). As determined by quasielastic light scattering, 1% (wt/vol) micellar concentrations of each unconjugated bile salt studied herein completely solubilized 1% (wt/vol) insulin by forming mixed micelles of bile salt and insulin molecules. When compared with the identical bile salt solutions without insulin, the degree of micellar expansion induced by insulin corresponded to micellar solubilized insulin monomers (unpublished observations). Mixed micelle formation most likely provides a high juxtamembrane concentration (in our formulations, ≈1–5 mM) of soluble insulin that facilitates the flow of insulin monomers down a concentration gradient from the nares into the nasal membranes. These considerations are the most likely explanation for the dose–response curve in Fig. 2, where bile salt adjuvant activities became unmasked only above the aqueous cmc of the deoxycholate and leveled off at concentrations customarily accepted as corresponding to well-developed stepwise self-association (34).

Because 1% wt/vol of each bile salt completely solubilized 1% wt/vol insulin as monomers, we believe, on the basis of other studies (unpublished observations, this paper), that differing adjuvant activities of various bile salt species relate to their differing capacities to penetrate and self-associate as reverse micelles within native membranes (hydrophobic bile salts >> hydrophilic bile salts) as they do in nonpolar solvents or when dispersed in pure phospholipid environments (10, 19, 33). In reverse micelles, the hydrophilic surfaces of the molecules face inwards and the hydrophobic surfaces face outward toward the lipid environment (Fig. 3). Thus, reverse micelles could act as transmembrane channels or mobile carriers for insulin to move down an aqueous concentration gradient through the nasal mucosal cells, into the intercellular space, and into the blood stream (Fig. 3). While this mode of intramembranous bile salt self-association has been invoked to explain the high solubility of bile salts in membranes and synthetic bilayers (10, 19), its functional significance has only recently been addressed. Hunt (43) and Castallino and Violand (44), employing NMR spectroscopy, have suggested that reverse micelles of bile salt molecules account for the rapid transport of lanthanides into unilamellar vesicles, and Hunt (43) suggested, on the basis of kinetic analysis, that transmembrane diffusion of lanthanide/bile salt complexes occurred. However, our study and that of Murakami et al. (17) show transport saturation at bile salt concentrations of ≈12 mM (Fig. 2). This finding argues for local membrane saturation with the steroid detergent and a channel-type reverse micelle mechanism rather than a mobile reverse micelle carrier. The far greater adjuvant potency of the more hydrophobic dihydroxy bile salts with two α-oriented hydroxyl functions compared with cholate and ursodeoxy-

![FIG. 2. Dependence of peak serum insulin on concentration of sodium deoxycholate. Peak increment in serum insulin is plotted versus the final concentration of sodium deoxycholate in the nasal spray. The equivalent of 1% (wt/vol) sodium deoxycholate is 24 mM. Insulin was administered at a dose of 0.5 U/kg. (Inset) Kinetics of insulin absorption at three different concentrations of sodium deoxycholate. Results are presented as mean ± SEM.](image-url)
channels of a cubic phase formed by monoolein/water systems. Finally, Hirai et al. (23) have suggested that bile salts may promote insulin transport across the nasal mucosa by retarding insulin degradation by leucine aminopeptidase, a proteolytic enzyme of the nasal mucosa. When a 20-fold excess of tyrosyltyrosine, an alternative substrate for this enzyme was added, the dipeptide did not influence the extent of insulin absorption from the bile salt nasal spray (unpublished observations). Obviously, more work on the mechanisms involved in transmembrane transport of insulin by bile salts is needed.

A prime objective in developing an adjuvant for the transmucosal administration of insulin and other polypeptides is to identify an effective membrane-homing surfactant that does not cause local or systemic toxicity. Each of the unconjugated bile salts tested in this study produced local nasal irritation as assessed by a brief (3- to 5-min) burning sensation in the nose. However, this irritation did not correlate with the adjuvant activity of the bile salt, since ursodeoxycholate, which was inactive, was the most irritating. Taurine and glycine conjugates of the bile salts were somewhat less irritating to the nasal mucosa, as they are in terms of toxicity on other membranes (7). The observation that adjuvant activity of bile salts for transmembrane insulin transport can be predicted on the basis of the hydrophilic–hydrophobic balance of the monomers, a property easily measured (24), raises the possibility that bile salts or related molecules can be structurally engineered to retain adjuvant activity without producing potentially toxic side effects.

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Medical Sciences: Gordon et al.