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Report

Site Dependence of Absorption-Promoting Actions of Laureth-9, Na Salicylate, Na_2EDTA, and Aprotinin on Rectal, Nasal, and Buccal Insulin Delivery

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The site dependence of the absorption-promoting actions of laureth-9, Na salicylate, Na_2EDTA, and aprotinin was studied in rats. Insulin absorption was estimated on the basis of the cumulative hypoglycemic response from 0 to 4 hr postdose, relative to that after intramuscular insulin. Insulin was administered with or without adjuvants to isolated rectal, nasal, and buccal absorption sites. Laureth-9, a nonionic surfactant which irreversibly removes membrane proteins or lipids, promoted insulin absorption from each site. The rectal, nasal, and buccal routes were 30% as effective as the i.m. route. The enhancing effects of Na salicylate and Na_2EDTA, which have reversible mechanisms of permeability enhancement, were specific for rectal absorption. With these adjuvants, rectal insulin was 30–40% as effective as i.m. insulin, but nasal and buccal doses were less than 5% as effective as i.m. doses. This specificity can be at least partly explained by considering the site-to-site differences in membrane histology, although differences in pore size and membrane biochemistry might also contribute. The protease inhibitor aprotinin was ineffective in increasing insulin efficacy via each route, either alone or in combination with laureth-9.

KEY WORDS: absorption promoters; insulin; membrane penetration; buccal; nasal; rectal.

INTRODUCTION

Adjuvants that increase membrane permeability have been used to promote the absorption and increase the bioavailability of poorly absorbed drugs, particularly proteins and certain antibiotics. Because the drug and adjuvant must be coadministered to an absorption site of restricted area, the most feasible uses of adjuvant/drug combinations are for rectal, nasal, and buccal or sublingual delivery. Delivery via these sites would be an attractive alternative to frequent injections if sufficient absorption could be achieved. Although various alternative dosing routes have been proposed, there have been few studies comparing these routes and addressing the advantages and disadvantages of each. One of the primary criteria to compare is the relative absorption from the rectal, nasal, and oral mucosae and the effects of absorption promoters on each route. In a previous study (1), we showed that insulin administered rectally, nasally, buccally, or sublingually was much less effective than intramuscular insulin, if an absorption promoter was not coadministered, but Na glycocholate significantly improved absorption from each site (1). In this study we compared the effects of Na salicylate, Na_2EDTA, and laureth-9 on insulin absorption via the rectal, nasal, and buccal routes.

These adjuvants promote absorption by increasing membrane permeability. Several mechanisms for permeability enhancement have been proposed. Surfactants such as laureth-9 damage membranes by dissolving their lipids and/or proteins (2,3). In contrast, it is thought that Na_2EDTA affects the tight junctions interconnecting membrane cells and consequently increases paracellular or pore transport (4,5). Na salicylate, it was suggested, interacts with membrane proteins (6) and reduces the levels of membrane nonprotein thiols (7) to increase transcellular absorption, and may also increase paracellular transport by calcium chelation (8). Na_2EDTA and Na salicylate have been used mostly to promote rectal and intestinal membrane permeability. Their effects on nasal and buccal absorption have not been described. Since these adjuvants presumably have different mechanisms of increasing membrane permeability, and because the morphologies of the rectal, nasal, and oral mucosal membranes differ, the effects of absorption promoters might be expected to vary from site to site. The rectal mucosal membrane is a simple columnar epithelium: a single cell layer with tight junctions forming intercellular contact points. In contrast, the buccal and sublingual membranes are stratified squamous epithelia which are keratinized in some areas. Tight junctions are rare, and the barrier to paracellular transport is derived from the membrane coating granules (9). Therefore, if adjuvants act exclusively at the tight junctions, their actions on buccal absorption might not be as great as on rectal absorption. This hypothesis was tested.

Bioavailability of proteins administered via mucosal membranes can also be improved by inhibiting metabolism

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of the proteins at the absorption site. The protease inhibitor aprotinin has been shown to improve subcutaneous (10) and intestinal (11) insulin absorption, but the effects of protease inhibitors at various mucosal absorption sites have not been compared. Therefore, we also evaluated the effects of aprotinin on rectal, nasal, and buccal insulin delivery.

MATERIALS AND METHODS

Crystalline bovine insulin, Na salicylate, Na_2EDTA (ethylenediaminetetraacetic acid, disodium salt, dihydrate), laureth-9 (polyoxyethylene 9 lauryl ether), and aprotinin (lyophilized powder from bovine lung) were obtained from Sigma Chemical Company. Dosing solutions were prepared by dissolving Na salicylate, Na_2EDTA, or laureth-9 in 0.1 *M* phosphate buffer and adjusting the pH to 7.4. Insulin (23.4–25 U/mg activity) was then added to give concentrations of 10 to 250 U/ml. Solutions were warmed to approximately 40°C to dissolve the insulin. Dosing solutions containing Na salicylate, laureth-9, or no adjuvant were clear. Solutions containing Na_2EDTA were clear at 40°C but became cloudy at room temperature. For dosing with aprotinin, the insulin solution was prepared as above, and then aprotinin was added. The aprotinin activity was 11 to 12 TIU (trypsin inhibitor units)/mg and the dose administered was 54 U/kg (270 U/ml). Separately, insulin and aprotinin were soluble, but the final dosing solution containing the mixture was turbid.

Male Lewis rats (Charles River, Kingston, N.Y.) weighing 275–325 g were fasted for at least 16 hr prior to dosing. Insulin was administered either rectally, nasally, or buccally. In each case the dosing site was isolated surgically (ether anesthesia) 1.5 to 2.5 hr prior to dosing. These methods were reported in detail previously (1). For nasal dosing the trachea was cannulated to allow free breathing and the posterior nasal cavity was plugged via the esophagus. The incisive ducts were sealed with cyanoacrylic adhesive, and the dosing solution was injected through the nares. For buccal dosing the esophagus was ligated to prevent swallowing of the dosing solution. For rectal dosing, the rectum was isolated using two silicone rubber septa (0.7-cm diameter) connected by a 1-cm length of galvanized metal wire. Dosing was by injection through the posterior septum, which was glued to the anus with cyanoacrylic adhesive. Rats dosed rectally also had the esophagus ligated prior to dosing so that the initial plasma glucose concentrations would not be different from those in rats dosed nasally and buccally. All dosing volumes were 0.2 ml/kg, and the solutions did not visibly leak from the site of administration. The animals were allowed to recover from surgery for 1.5 to 2.5 hr. They were then anesthesized with 700 mg/kg urethane i.p. An initial blood sample was taken and rats were administered insulin. Rats were then restrained in a prone position. The anesthesia and restraint were intended to reduce movement to minimize loss of the dosing solution from the absorption site. Serial blood samples (0.3–0.4 ml) were collected by cutting the tip of the tail. Blood was collected in tubes containing heparin; plasma was separated and frozen. Plasma glucose determinations were made on an autoanalyzer, using a method based on the phosphorylation of glucose by hexokinase.

The hypoglycemic response provides a measure of the extent of insulin absorption. Plasma glucose concentrations were expressed as the percentage change from the initial (predose) concentration. The area under the percentage change vs time curve from 0 to 4 hr represents the cumulative percentage change. A negative value of cumulative percentage change reflects a net decrease in plasma glucose. These values were then used to calculate how effective rectal, nasal, and buccal insulin were relative to intramuscular insulin using an i.m. dose/response curve as previously reported (1). The equation for that curve was

$$\text{dose}^* = 10^{\left(\frac{\text{cumulative \% change} + 166.579}{-140.862}\right)}$$

where cumulative % change represents the values for rectal, nasal, or buccal insulin, and dose* is the equally efficacious i.m. dose. Then

$$\text{efficacy relative to i.m. (\%)} = \frac{\text{dose}^*}{\text{dose}^{\text{rectal, nasal, buccal}}} \times 100$$

The value of percentage efficacy relative to i.m. was calculated for each rat. If the value of the cumulative percentage change was positive (glucose increased), the percentage efficacy was zero. For this method to be valid, the measured hypoglycemic response had to be within the linear range of the log dose/response curve. The efficacy of any route relative to that of the i.m. route is similar, but not necessarily equivalent, to the bioavailability, which would be based on ratios of areas of plasma insulin concentration vs time.

RESULTS AND DISCUSSION

We previously showed that the hypoglycemic response (area under the plasma glucose percentage change vs time curve from 0 to 4 hr) to intramuscular insulin was linearly related to the logarithm of the dose, within a limited response range (1). The hypoglycemic response to insulin administered by other routes can be used to estimate how effective those routes are, relative to the intramuscular route, as long as the response is within the range of linearity. In some groups different doses were administered so that a valid calculation of the efficacy relative to i.m. could be made. There were no significant differences (by analysis of variance or *t* tests) within the groups given rectal or buccal insulin at various doses without an absorption promoter. These percentage efficacy values were therefore averaged to give a single control-group value for each route (see Table I). Nasal and buccal insulin were quite ineffective relative to i.m. insulin, if no absorption promoter was coadministered.

Absorption promoters were evaluated by each route. Results with laureth-9 and Na salicylate are illustrated in Fig. 1 and all data are summarized in Table I. Laureth-9 significantly promoted insulin absorption from each site, and efficacy values for all routes were approximately equal. Promoting effects of laureth-9 on rectal (12) and nasal (13) insulin absorption were previously reported by others. In contrast to laureth-9, Na salicylate had no significant promoting effect on nasal or buccal insulin absorption but significantly improved rectal insulin efficacy. Similarly, Na_2EDTA had no significant promoting effect on nasal and buccal insulin absorption but was as effective as laureth-9 in promoting rectal absorption. Both Na salicylate (14) and Na_2EDTA (15) were

Table I. The Effects of Adjuvants on Rectal, Nasal, and Buccal Insulin Efficacy Relative to Intramuscular Insulin Efficacy

Adjuvant	Insulin dose (U/kg)	Efficacy relative to i.m. (%)[a]		
		Rectal	Nasal	Buccal
None	2	10.8 ± 3.1		
	10	17.0 ± 5.6	0.4 ± 0.2[b]	3.6 ± 2.8
	20	7.0 ± 1.4		
	50	3.2 ± 0.6	2.0 ± 0.5	0.7 ± 0.3
	Avg. all doses	9.5 ± 2.0		1.9 ± 1.3
Laureth-9 (5%)	10	31.9 ± 11.0*	28.7 ± 6.9*	27.2 ± 10.3*
Na salicylate (5%)	10	41.7 ± 11.3*	4.1 ± 2.4	2.9 ± 2.0
	50			1.0 ± 0.7
Na_2EDTA (5%)	10	31.0 ± 6.7*	3.5 ± 1.0	0.9 ± 0.4[b]
	50			2.9 ± 1.4
Aprotinin (270 U/ml)	10	15.1 ± 2.8	9.6 ± 4.3	2.4 ± 1.6
Aprotinin + Laureth-9	5	25.7 ± 7.0*	13.0 ± 1.8	5.7 ± 2.8

[a] Mean ± SE of six or more animals per group.
[b] Mean response was lower than the lowest point on the i.m. dose/response curve; group not used in statistical comparisons.
* Significantly ($P < 0.05$) different from control, by t test.

previously shown to enhance rectal insulin absorption. Neither had been studied as an enhancer for nasal or buccal delivery, however. Clearly, when Na salicylate or Na_2EDTA was used as an adjuvant the rectal route provided greater insulin efficacy than the nasal and buccal routes.

Laureth-9, Na salicylate, and Na_2EDTA are thought to promote mucosal membrane permeability by different mechanisms. Surfactants irreversibly increase permeability by extracting proteins or lipids from the membrane (2,3). Na_2EDTA increases paracellular transport by affecting the permeability of the tight junctions connecting cells as a consequence of the removal of luminal calcium (4,5). Na salicylate apparently interacts with membrane proteins (6) and nonprotein thiols (7) to increase transcellular transport, but unlike surfactants the effect is reversible. Na salicylate may increase paracellular transport as well (8). The data presented here indicate that the rectal, nasal, and buccal membranes have different levels of susceptibility to these absorption-promoting effects. The lack of susceptibility of the buccal membrane to promotion of paracellular transport (as with Na_2EDTA) is expected, since the membrane structure is a stratified, squamous epithelium lacking tight junctions (9). The nasal mucosa consists of several different types of epithelia (see Ref. 16). The vestibule, the most anterior portion of the nasal cavity accounting for only 3–4% of the total surface area, is lined with stratified squamous epithelia. The respiratory epithelium is comprised of ciliated cuboidal and columnar cells and goblet cells. The olfactory epithelium is a pseudostratified neuroepithelium. The permeability characteristics of these diverse epithelia have not been compared, and we have not determined how a nasal dose distributes within the nasal cavity in rats. Although the rectal and nasal

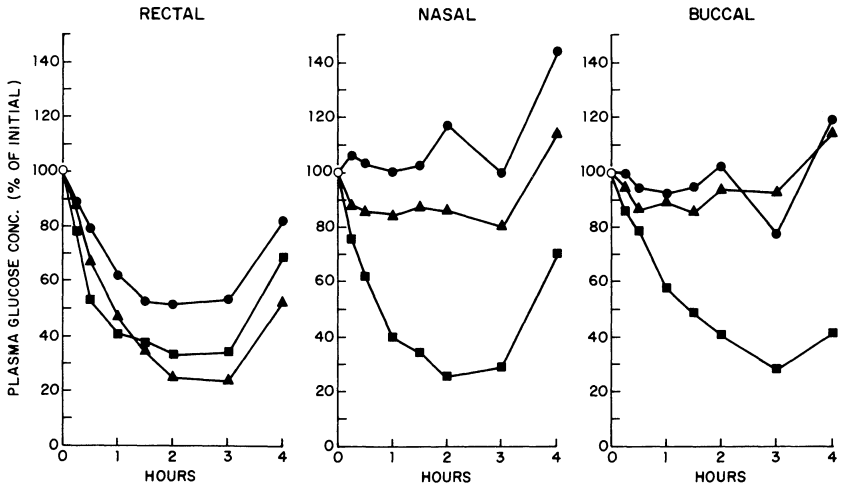

FIG. 1. Average plasma glucose concentrations in rats administered rectal, nasal, or buccal insulin (10 U/kg) with no adjuvant (●) or with laureth-9 (■) or Na salicylate (▲).

mucosae are at least in part structurally similar, having columnar epithelia, differences in permeability and susceptibility to permeability enhancement are apparent. One possible explanation is that the pores of the nasal mucosa, even when the tight junction barrier is compromised, may be too small for insulin to pass through. Nakanishi *et al.* (15) showed that the enhancing effects of Na_2EDTA on rectal permeability decreased as the molecular weight of the absorbant increased. Hayashi *et al.* (17) previously suggested that the rat nasal membrane has a richer distribution of water channels than the rectal membrane but has a smaller pore size. It could also be proposed that the tight junctions of the nasal mucosa are not susceptible to the effects of Na_2EDTA. The specificity of Na salicylate effects on the rectal mucosa also suggests morphological or biochemical differences among the rectal, nasal, and buccal mucosae, although the nature of these differences is not known.

Even with absorption promoters, rectal, nasal, and buccal insulin was less than half as effective as i.m. insulin. Metabolism at the absorption site could contribute to this loss in efficacy. Then, just as there are differences among absorption sites in membrane permeability and in the effects of adjuvants on permeability, the extent of metabolism of insulin could vary from site to site. In fact, differences in rates of insulin degradation by rabbit membrane homogenates were reported; insulin degradation half-lives were 88 min for rectal, 169 min for nasal, and 297 min for buccal mucosae (18). Protease inhibitors such as aprotinin could therefore have site-dependent effects on insulin absorption. In our studies, when aprotinin was used as the sole adjuvant, nasal insulin efficacy was slightly, but not significantly, improved. Rectal and buccal insulin with aprotinin was no more effective than in the absence of an adjuvant (Table I). Nishihata *et al.* (19) also showed that aprotinin alone had no effect on rectal insulin absorption in rats, but aprotinin combined with Na salicylate increased insulin absorption more than Na salicylate alone. Aprotinin itself may not have been absorbed or been accessible to the insulin-metabolizing enzymes, because of its fairly high molecular weight (6512). Therefore, combinations of aprotinin and laureth-9 were examined; however, no additional absorption-promoting effect, compared to laureth-9 alone, was observed. Nasally and buccally, the combinations of laureth-9 and aprotinin were less effective than laureth-9 alone. The effects of laureth-9 on aprotinin activity and the physiochemical interactions of laureth-9, insulin, and aprotinin are not known. Therefore, conclusions cannot be drawn from these data regarding the importance of insulin metabolism at these absorption sites.

In summary, the rectal, nasal, and buccal mucosae exhibited different susceptibilities to the effects of membrane penetration enhancers for insulin. The nonionic surfactant laureth-9 promoted absorption from each route, and the efficacy from each route was approximately 30% of the intramuscular efficacy. This is similar to the effects of Na glycocholate on insulin absorption from each route (1). Surfactants and bile salts are generally considered to be damaging to membranes. Na salicylate and Na_2EDTA, which the literature suggests have different mechanisms than bile salts and surfactants in promoting absorption, selectively increased rectal permeability. In considering routes for protein drug delivery, the rectal route apparently has the advantage of not requiring membrane-damaging agents for use as absorption promoters.

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