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The influence of concentration of two salicylate derivatives on rectal insulin absorption enhancement

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Abstract—3,5-Diiodosalicylate sodium (DIS), a highly lipophilic salicylate, was evaluated against 5-methoxysalicylate sodium (MS) as a potential adjuvant absorption promoter for rectal insulin delivery. Comparative blood glucose measurements were made using the two adjuvants under identical conditions as promoters of rectal insulin absorption in rats. Concentrations of DIS greater than and including 0·1 M produced an unexpected, progressive decrease in adjuvant activity as determined by a decline in observed hypoglycaemic response. This was not due to formation of an insulin-DIS complex. The adjuvant MS produced a classical, sigmoidal log-dose response curve. Possible reasons for the occurrence of the DIS optimum phenomenon are discussed as well as are the observed differences in adjuvant potency of these agents in a propylene glycol-containing vehicle.

Salicylate analogues can enhance the absorption of insulin through the gastrointestinal epithelial mucosa by a reversible and apparently non-damaging mechanism (Nishihata et al 1981, 1983; Nakanishi et al 1984).

Structure-activity studies on the effects of salicylate analogues on membrane ion permeability demonstrated that their relative potencies are correlated with their octanol:water partition coefficients and pK_a values (Levitan & Barker 1972).

We have sought to determine if the same factors governing the relative membrane ionic permeability activity of salicylates could be related to their adjuvant potency as absorption promoters for the rectal delivery of insulin.

Materials and methods

Chemicals. Insulin human injection, USP (Eli Lilly, Indianapolis, IN) was used. The 5-methoxy- and 3,5-diiodo-analogues of salicylic acid were obtained from Aldrich Chemical Company, Milwaukee, WI, and converted to the sodium salts by titration with sodium hydroxide in absolute ethanol (Levitan & Barker 1972).

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Preparation of microenema solutions. Microenema solutions were freshly prepared by dissolving the appropriate amount of adjuvant in a vehicle consisting of 0·2 M phosphate buffer (pH 5) containing 50% propylene glycol, which was necessary to solubilize DIS. Insulin was then added to a final concentration of 6 iu mL^{-1} of solution.

Insulin absorption studies. Male Sprague-Dawley rats (200–300 g) from Taconic Farms (Germantown, NY) were fasted for 20 h before experiments for which they were anaesthetized with pentobarbitone sodium (50 mg kg^{-1}). The right jugular vein was cannulated with 0·020-inch i.d. silastic tubing (Dow-Corning Corporation, Midland, MI) inserted to about 3·2 cm past the clavicle and secured with 3–0 silk thread; the cannula was flushed with heparin in 0·9% NaCl (5 units mL^{-1}) (Grasela & Rocci 1984). The animals were placed ventral-side down and microenemas, 1 mL kg^{-1} administered at a depth of 1 cm from the anus which was ligated with thread (Nishihata et al 1984). Serial blood samples (100 μL) were drawn immediately before and at 15, 30, 60, 90, and 120 min following the microenema. Blood was not replaced. Blood samples were immediately transferred to 1·5 mL polypropylene tubes—heparinized by adding 20 μL of a 20 units mL^{-1} solution to the tubes and permitting it to evaporate. All samples were stored over ice until assay on the same day of the experiment. Immediately before assay, blood samples were warmed to 25°C.

Glucose determinations were made on 20 μL amounts of blood using a commercially available kit (Accu-Check II, Boehringer Mannheim Diagnostics Division, Indianapolis, IN).

Insulin-DIS binding study. Binding of DIS by insulin to form a less bioavailable complex could possibly be responsible for the optimum phenomenon observed. We therefore evaluated the affinity of the insulin molecule for DIS by an ultrafiltration technique.

A 2·5 mL sample of a solution of biosynthetic human insulin (Eli Lilly, Indianapolis, IN) at 1 mg mL^{-1} and DIS 1·0 mM was prepared in a vehicle of 0·2 M phosphate buffer (pH 5) and 50% propylene glycol. A control solution similarly prepared con-

tained no insulin. Four 500 μL samples of each solution were subjected to ultrafiltration using Amicon MPS-1 ultrafiltration units equipped with 1000 Dalton cut-off filters. Centrifugation was at 4000 rev min^{-1} (2060 *g*) for 2·5 h at 25°C in a Beckman J2-21 refrigerated centrifuge equipped with a JA-20.1 fixed-angle rotor. Relative concentrations of DIS in the ultrafiltrates and corresponding unfiltered solutions were determined by HPLC assay on a 25 cm C_{18} reversed phase column; mobile phase 0·01 M phosphate buffer (pH 7·4)-methanol (45:55 v/v); flow rate 1·5 mL min^{-1}. Detection was by uv at 313 nm.

Results

Insulin absorption studies using MS and DIS. A close relation between plasma glucose concentration and insulin bioavailability has been shown by Nishihata et al (1981, 1985). We determined the relative ability of the adjuvants DIS and MS to enhance insulin absorption from the maximum percent drop in blood sugar from initial value (E_{max}) (Touitou & Rubinstein 1986). Fig. 1 shows the E_{max} vs log concentration dose response curves for DIS and MS. Mean E_{max} values for corresponding concentrations of adjuvant were subjected to ANOVA and significant differences ($P<0·05$) identified with Duncan's New Multiple Range Test. The decrease in the DIS E_{max} from its peak

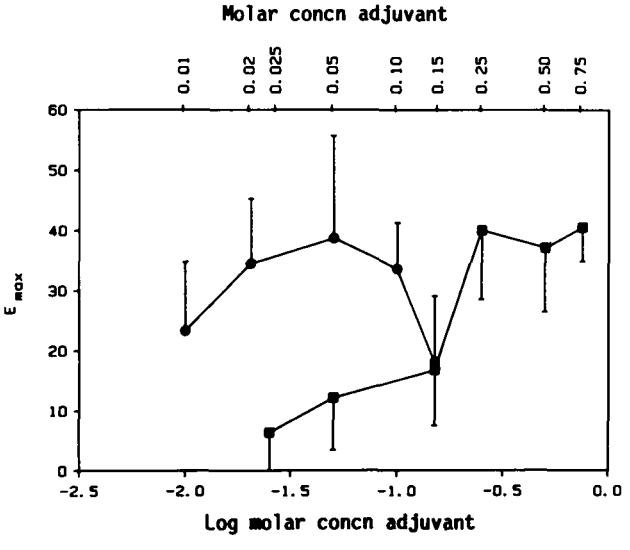

FIG. 1. Hypoglycaemic effect of insulin administered rectally to rats in 0·2 M phosphate buffer (pH 5)/propylene glycol (50:50 v/v) containing: ● DIS; ■ MS. The error bars represent the standard deviation with $n \geq 6$; $n = 4$ for 0·75 M MS.

value at 0·05 M is statistically significant ($P<0·05$) at 0·15 M. When MS was used as the adjuvant, peak E_{max} values were observed after 0·25 M. Concentrations of MS higher than 0·25 M produced comparable effects ($P<0·05$).

Control experiments were run on vehicle solutions containing insulin 6 iu mL^{-1} and no adjuvant. A second control was run on vehicle solutions containing 0·15 M DIS and no insulin to rule out the possibility that an adjuvant-induced hyperglycaemia was responsible for the decreased hypoglycaemic effect seen at that concentration of DIS. No significant difference in blood sugar values between those two controls was seen nor was there any significant change in blood sugar from the initial value during either control experiment ($P<0·05$, $n=3$).

Relative potency determination for MS and DIS was made by constructing parallel lines over the linear portions of the log dose-response curves and taking the antilog of the distance between these lines (Tallarida & Murray 1984). DIS was found to possess an adjuvant potency 14 times greater than MS.

Effects of propylene glycol on adjuvant activity of MS and DIS. For this purpose, concentrations of 0·02 M DIS and 0·25 M MS were chosen since they elicited comparable E_{max} values. For DIS, the adjuvant effect for a vehicle without propylene glycol ($E_{max} = 34·6 \pm 6·7$) was not significantly different ($P<0·05$) from a propylene glycol-containing vehicle ($E_{max} = 34·5 \pm 10·8$). For 0·25 M MS in a propylene glycol-free vehicle ($E_{max} = 22·1 \pm 10·5$), however, a significant decline ($P<0·05$) in adjuvant activity was observed when compared with a propylene glycol-containing vehicle ($E_{max} = 40·0 \pm 11·5$).

Insulin-DIS binding study. The relative concentrations (μg mL^{-1}) of 1·0 mM DIS in vehicle-only and in a vehicle containing insulin were: vehicle (control) $365 + 18·5$ (filtered), $405 \pm 10·3$ (unfiltered); vehicle & insulin $335 \pm 17·3$ (filtered), $389 \pm 7·1$ (unfiltered). Concentrations of DIS in filtered insulin-containing samples were not significantly lower than filtered vehicle-only samples ($P<0·05$, $n=4$). The lower concentration of DIS in the ultrafiltrates is attributed to binding of salicylate to the filter membrane (Moran & Walker 1968).

Discussion

We have demonstrated that the ranking of two salicylate analogues for the relative magnitude of their effects on membrane ion permeability (Levitan & Barker 1972) is related to their potencies as adjuvant absorption promoters for insulin which appear to be related to their hydrophobicities. DIS has an octanol:water partition coefficient 234 times greater than MS (Levitan & Barker 1972) and possessed 14 times greater adjuvant potency. This is consistent with McLaughlin's (1973) studies, in which he concluded that the binding of salicylates to lipid bilayers involved primarily hydrophobic forces, and the results of Nishihata et al (1982a), which showed a relation between adjuvant potency and degree of binding to the rectal membrane.

The occurrence of an optimal concentration of DIS for the promotion of rectal insulin absorption may also be related to its relatively high degree of membrane binding. Adsorption of salicylate anion to bilayer lipid membranes has been found to depress the conductance due to negative permeant molecular species (McLaughlin 1973). Higher concentrations of DIS would be expected to result in an increased adsorption of this adjuvant to the rectal membrane with a resultant drop in absorption of the negatively charged insulin molecules (McLaughlin 1973). However, more recent work concerning the mechanism by which salicylates enhance membrane permeability suggests that increased membrane binding of salicylates would result in greater membrane perturbation with a resultant increase in permeability (Kajii et al 1985).

The occurrence of the DIS optimum phenomenon could also be explained by the work of Nadai et al (1976) which demonstrated a decrease in the lymph:blood absorption ratio of sulphanilic acid from the rat small intestine when adjuvant concentration was increased. Higher concentrations of the adjuvants EDTA and tetracycline were associated with a net decrease in drug absorption via the lymphatic route which could explain the decrease in hypoglycaemic effect seen at the higher concentration of DIS, as intestinal insulin absorption occurs primarily via the lymphatic pathway (Nishihata et al 1982b).

The apparent co-adjuvant activity of propylene glycol and the more water-soluble MS may be due to the ability of salicylates to adjust their solubility characteristics in accordance with solvent polarity (Martin et al 1985). The addition of propylene glycol reduces the dielectric constant of the vehicle. This may encourage more intramolecular hydrogen bonding (Sloan et al 1986) and increase the apparent pK_a of MS (Stewart 1966). Both of these effects enhance the lipophilic character of the molecule and

favour its penetration into the predominantly hydrophobic rectal membrane. An increase in adjuvant activity would be the expected result (Kajii et al 1986).

The similar adjuvant activity demonstrated by DIS in both vehicles would appear to be due to its exceptionally high octanol:water partition coefficient which favors its association with the predominantly lipophilic rectal membrane regardless of the presence of the propylene glycol co-solvent.

Binding of salicylates by plasma proteins is a well established phenomenon (Moran & Walker 1968; Spector et al 1972). Binding of salicylate by human serum albumin is believed to occur via electrostatic interaction with the cationic centres of the albumin molecule, specifically the free amino groups (Lindenbaum & Schubert 1956). Each human insulin molecule contains six free amino groups which would exist in a cationic state at pH 5. The theoretical maximum binding to these sites would be 427 μg of DIS per mg of insulin, which formed the theoretical basis for our selection of the DIS concentration employed in the binding study. The absence of a significant difference in ultrafiltrate concentrations of DIS in the insulin-containing vs control solutions has led us to conclude that, under the conditions of our study, binding of DIS by the insulin molecule is negligible. Thus, based on these results, we do not feel that complex formation between insulin and DIS is responsible for the optimum phenomenon.

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