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Research Article

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Modulation of Intestinal Tight Junctions by Zonula Occludens Toxin Permits Enteral Administration of Insulin and Other Macromolecules in an Animal Model

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Abstract

The intestinal epithelium represents the major barrier to absorption of orally administered drugs and peptides into the systemic circulation. Entry of molecules through the paracellular pathway is restricted by tight junctions. We have previously reported that these structures can be modulated by Zonula occludens toxin (Zot). In the present report, we show that Zot reversibly increases rabbit intestinal permeability to insulin by 72% ($P = 0.034$) and immunoglobulins by 52% ($P = 0.04$) in vitro. When tested in vivo, Zot induced a 10-fold increase of insulin absorption in both the rabbit jejunum and ileum, whereas no substantial changes were detected in the colon. Similar results were obtained with immunoglobulins, whereby Zot induced twofold and sixfold increases of IgG absorption in the jejunum and ileum, respectively. In diabetic rats, bioavailability of oral insulin coadministered with Zot was sufficient to lower serum glucose concentrations to levels comparable to those obtained after parenteral injection of the hormone. The survival time of diabetic animals chronically treated with oral insulin + Zot was comparable to that observed in parenterally treated rats. These studies offer an innovative strategy for the oral delivery of drugs and proteins normally not absorbed through the intestine. (*J. Clin. Invest.* 1997. 99:1158–1164.) Key words: permeability • oral delivery • drugs • diabetes • immunodeficiency

Introduction

Intestinal absorption of numerous compounds routinely used for the treatment of common diseases is profoundly limited by their physicochemical characteristics. With the exception of those molecules which are transported by active or facilitated transcellular mechanisms, absorption of large and more hydrophilic macromolecules is almost exclusively limited to the paracellular pathway (1). Under normal conditions, however, this pathway is restricted to molecules with molecular radii < 11 Å (2). The utility of the paracellular route for oral drug delivery has remained unexplored due to a limited understanding of tight junction (tj)[1] physiology and the lack of substances capable of increasing the tj permeability without irreversibly compromising intestinal integrity and function (3–6). We have recently demonstrated that Zonula occludens toxin (Zot), a protein elaborated by *Vibrio cholerae* (7, 8), induced modifications of cytoskeletal organization that lead to the opening of tj related to the PKCα-dependent polymerization of actin monomers into actin filaments strategically localized to regulate the paracellular pathway (9). We have also shown that this modulation is reversible, time- and dose-dependent, and is confined to the small intestine, since Zot does not affect the colon permeability (7, 10). The selective effect of the toxin on the small intestine seems related to the regional distribution of the Zot receptors that are present in the jejunum and ileum, but not in the colon (10). The aforementioned properties make this moiety a potential tool for modulating the tj permeability. In the present report, we have demonstrated that Zot may be used to enhance the intestinal absorption of orally administered macromolecules through the paracellular pathway.

Methods

Zot purification. Purified Zot was obtained as previously described (10). Briefly, *zot* gene was fused in frame with the maltose binding protein (MBP) *malE* gene, using vector pMal-c2 (10) to create a MBP-Zot fusion protein. The fusion product was expressed in *Escherichia coli*, and obtained by disrupting the cells using a french press. The MBP-Zot fusion protein was then purified by affinity chromatography using an amylose column (MBP-fusion purification system, New England Biolabs, Beverly, MA). Purified Zot was finally obtained by cleaving the fusion protein with factor Xa (10). The amount of toxin obtained was assessed by the Bradford method (11).

In vitro Ussing chambers experiments. Experiments were carried out as previously described (12) following an experimental protocol approved by our institutional animal welfare committee. Briefly, adult male New Zealand white rabbits (2–3 kg) were killed by cervical dislocation. Segments of rabbit intestine (including jejunum, ileum, and colon) were removed, rinsed free of the intestinal content, opened along the mesenteric border, and stripped of muscular and serosal layers. Eight sheets of mucosa so prepared were then mounted in Lucite Ussing chambers (1.12 cm^2 opening), connected to a voltage clamp apparatus (EVC 4000 WPI, Sarasota, FL), and bathed with freshly prepared buffer containing (mM): NaCl, 53; KCl, 5; Na_2SO_4, 30.5; mannitol, 30.5; Na_2HPO_4, 1.69; NaH_2PO_4, 0.3; $CaCl_2$ 1.25; $MgCl_2$

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1. *Abbreviations used in this paper:* MBP, maltose binding protein; Rt, tissue resistance; tj, tight junctions; Zot, Zonula occludens toxin.

1.1; $NaHCO_3$, 25. The bathing solution was maintained at 37°C with water-jacketed reservoirs connected to a constant-temperature circulating pump and gassed with 95% O_2/5% CO_2. Potential difference (PD) was measured, and short-circuit current (Isc) and tissue resistance (Rt) were calculated as previously described (7). Once the tissues reached a steady state condition, paired tissues, matched on the basis of their resistance, were exposed luminally to either ^{125}I-insulin 10^{-11}M (2 μCi = 10^{-12} M) (Amersham Corp., Arlington Heights, IL) or 156.25 ng ^{125}I-IgG (1 μCi = 83.3 ng), alone or in the presence of 1.1×10^{-10} M Zot. 1-ml aliquot from the serosal side and 50 μl from the mucosal side were immediately obtained to establish baseline values. Samples from the serosal side were then collected at 20-min intervals for the following 80 min. The reservoirs were then emptied, washed twice with Ringer's solution, and refilled with fresh Ringer's solution containing only 10^{-11} M ^{125}I-insulin or 156.25 ng ^{125}I-IgG previously added to the chambers. Two further 1 ml samples from the serosal side of each chamber were finally collected at 20-min intervals to establish whether the effect of Zot on the permeability of the drugs tested was reversible.

In vivo perfusion experiments. Intestinal perfusion was carried out as previously described, with minor modifications (10, 13). More specifically, after a 24-h fast, 2.5–3 kg adult male New Zealand white rabbits were anesthetized with 50 mg ketamine per kg body weight, followed by injection of 7.0 mg xylazine per kg body weight i.m. Body temperature was kept at 37°C by a lamp. The abdominal cavity was opened by a midline incision, and three distinct segments of the intestine: (*a*) the proximal jejunum below the ligament of Treitz, (*b*) the distal ileum, and (*c*) the proximal colon, were cannulated in the same animal. A second cannula was placed 10–15 cm below each proximal cannula. The segments were rinsed free of intestinal contents with 0.9% (wt/vol) NaCl warmed to 37°C. The proximal cannulae were connected by a polyvinyl tube to a peristaltic pump (model WPI SP220 I; World Precision Instruments, Inc.), and the three segments were simultaneously perfused at a rate of 0.4 ml/min with the perfusion solution (10) containing either 143 pmol/ml of ^{125}I-insulin, or 8.33 ng/ml ^{125}I-IgG. All the solutions were made isotonic by adjustment with NaCl, and the pH was fixed at 7.4 by gassing with 95% O_2/5% CO_2.

Eluates were collected in 20-min aliquots from the distal cannulae. An initial equilibrium period of 30 min was allowed, followed by three consecutive 20-min collection periods for baseline measurement of the net transport of water and either insulin or IgG, in each segment studied. Subsequently, 1.1×10^{-10} M Zot in PBS was added to the perfusion solution, and a second perfusion period (30 min equilibration + 3× 20 min collection) was carried out. To establish whether the effect of Zot on intestinal permeability was reversible, a third perfusion period was performed with the same solution used in the first period. At the end of each perfusion period, the mesenteric vein draining each segment perfused was cannulated, and a blood sample was obtained to measure the amount of ^{14}C-PEG-4000 and the macromolecule tested (either ^{125}I-insulin or ^{125}I-IgG) that reached the bloodstream. At the end of the experiment, the animal was killed, and the segments perfused were isolated, measured, dried and weighed. Water absorption was calculated as previously described (10).

In vivo experiments in BB/Wor diabetic rats. Acute, type 1 diabetes mellitus male BB/Wor rats (12, 13) were obtained from the Department of Pathology of the University of Massachusetts. The animals were anesthetized with a mixture of ketamine (113.2 mg/kg body wt [bw]) and acepromazine (0.68 mg/kg bw). The jugular vein was cannulated as previously described (14) to allow frequent blood drawing. The rats were kept fasting overnight. The following day, the animals were sedated by methoxyflurane inhalation, and the esophagus was cannulated to place the tip of the rigid cannula in the gastric antrum. The rats then received, via the cannulated esophagus, one of the following treatments: (*a*) oral ultralente® human insulin (Humulin® U; range 5-30 U, Eli Lilly and Co., Indianapolis, IN) in 400 μl of $NaHCO_3$ solution (1.5 g/100 cm^3, pH 8.2) so as to neutralize the gastric acidity, (*b*) oral Humulin® U (range 5–30 U) plus purified Zot (range 1.1–4.4 × 10^{-10} mol) in 400 μl $NaHCO_3$ solution, (*c*) oral Zot 1.1×10^{-10} mol in 400 μl of $NaHCO_3$ solution, (*d*) parenteral Humulin® U (range 1.2–2.4 IU) (the dose was established on the basis of the animal weight and glucose level, following the animal supplier recommendations) plus oral $NaHCO_3$ solution (400 μl), or (*e*) oral $NaHCO_3$, 400 μl. The glucose level of each animal was assessed before treatment, and at 30 min intervals thereafter, using a micromethod assay (One Touch® II blood glucose meter; Lifescan Inc., Milpitas, CA). 30 min after the beginning of the study, the rats were allowed food. To avoid possible biases, each animal was scheduled to receive all five treatments listed above on different days. Since some animals died before the completion of the entire protocol, only some rats received all five treatments listed above. The casualties were not significantly related to any of the treatments received, but were mainly because of the mechanical occlusion of the jugular cannulation. The different groups of treatment were comparable in terms of animal body weight and baseline glycemic levels.

In another set of experiments, the diabetic animals were divided in three groups of treatment: (*a*) parenteral Humulin® U (dose range 0.8–1.6 IU), (*b*) oral Humulin® U 10 IU+ Zot 1.1×10^{-10} mol, and (*c*) oral Humulin® U 10 IU. The body weight, temperature, and blood glucose level (before treatment and 60 min thereafter) were daily monitored. Once the rats died, segments of the small and large intestine were isolated, fixed in paraformaldehyde 4%, and processed for light microscopy examination.

Statistical analysis. All values are expressed as means±standard error (SE). The analysis of differences was performed by Student's *t*-test for either paired or unpaired variates when applicable. For the experiments with BB/W or diabetic rats, the level of glucose among animals who received oral insulin alone was compared with those that received oral insulin + Zot by repeated measures analysis of variance. In addition, individual *t*-tests were applied to each post-administration timepoint. A P value < 0.05 was considered to be statistically significant.

Results

In vitro animal model

Insulin and immunoglobulin G (IgG) were selected as prototype molecules to be tested for oral delivery, based on the relative size and structure, biological activities, and therapeutic relevance of these proteins. Rabbit ileum was mounted in Ussing chambers, and in vitro transepithelial transport of ^{125}I-insulin and ^{125}I-IgG from the mucosal (i.e., intestinal lumen) to the serosal (i.e., bloodstream) boundaries in the presence and absence of purified Zot was measured. Zot (1.1×10^{-10} M) reversibly increased the intestinal absorption of both insulin (3.84±0.41 fmol/cm^2 per min vs. 6.60±1.4 fmol/cm^2 per min, untreated vs. Zot-treated tissues, respectively, $P = 0.034$, $n = 4$) (Fig. 1 *C*) and IgG (7.97±1.17 pg/cm^2 per min vs. 12.1±1.8 pg/cm^2 per min, $P = 0.04$, $n = 4$) (Fig. 1 *D*) in a time-dependent manner. Zot permeabilizing effect peaked at 80 min, and was completely reversible within 20 min from the withdrawal of the toxin from the Ussing chambers (see Fig. 1). This Zot-induced increase in absorption coincided with a reduction in tissue resistance (Fig. 1, *A* and *B*). No significant changes of Rt were observed in control tissues after 80 min incubation, however, the tissues exposed to insulin alone showed an increase of Rt above the baseline when the solutions were changed (see Fig. 1 *A*).

In vivo animal model

To study the effect of Zot in the intact host, we used the in vivo perfusion assay (13). We have recently demonstrated that the effect of Zot on tissue permeability is limited to the small bowel, and this distinct permeabilizing effect of the toxin coin-

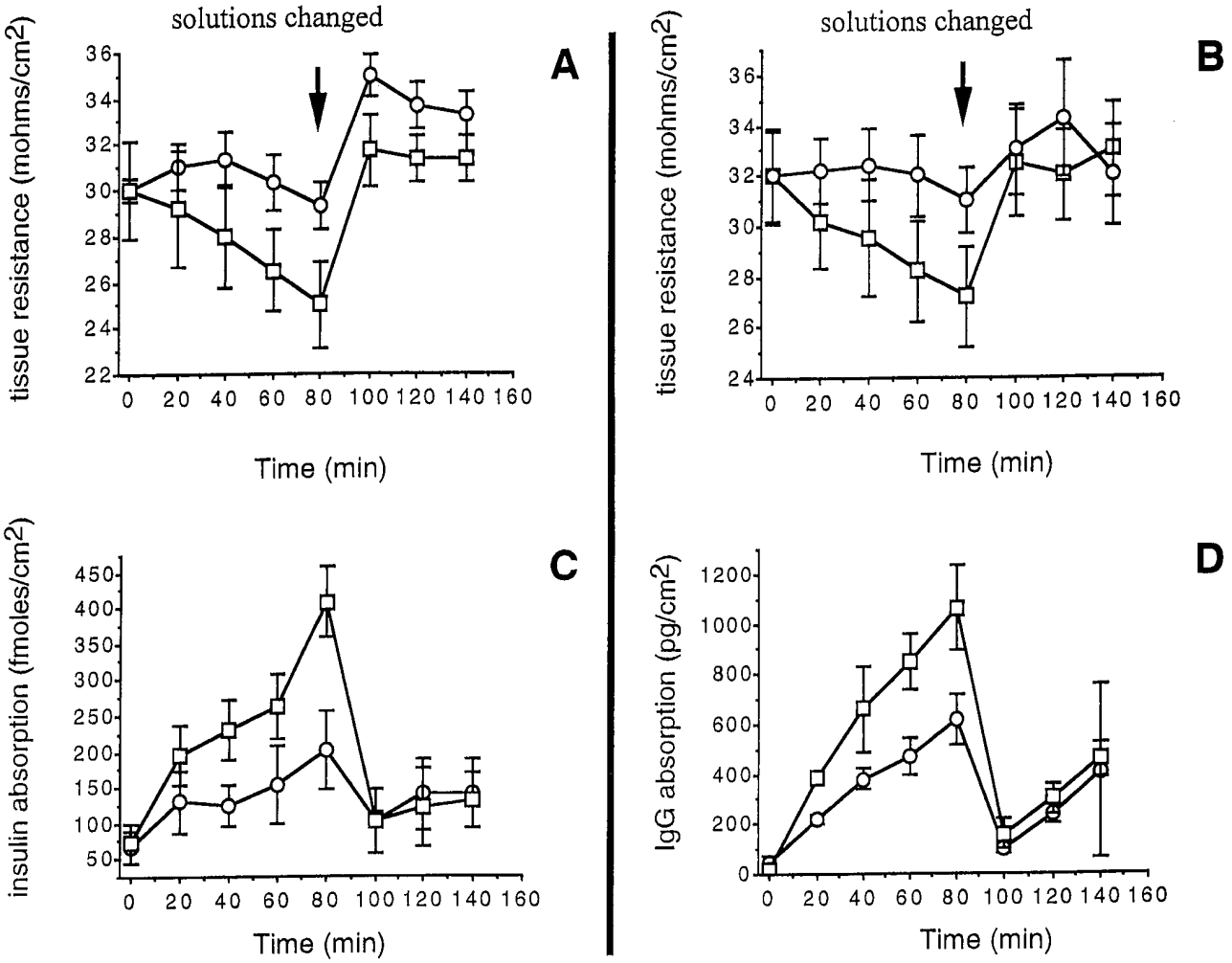

Figure 1. Reversible effect of purified Zot on tissue resistance (*A* and *B*) and transepithelial transport of insulin (*C*) and IgG (*D*) in rabbit ileum in vitro. Paired tissues, matched on the basis of their resistance, were exposed luminally to either 10^{-11} M ^{125}I-insulin (Amersham) (*left*) or 156.25 ng ^{125}I-IgG (*right*), alone (○) or in the presence of 1.1×10^{-10} M Zot (□). After 80 min of incubation, the Ringer's solutions were replaced with solutions of identical composition but without Zot. Zot reversibly increased the transepithelial absorption of both insulin and IgG. These effects paralleled the Rt decrement induced by the toxin. $n = 4$.

cides with the regional distribution of Zot receptor(s) within the intestine (10). Therefore, three distinct segments of rabbit intestine—the jejunum, the distal ileum, and the colon—were simultaneously perfused in the same animal. When added to the perfusion solution, Zot (1.1×10^{-10} M) increased the passage of ^{125}I-insulin across both the jejunum and distal ileum 10-fold, whereas no substantial changes were observed in the colon (Fig. 2). The increased absorption of insulin was reciprocal with a shift of water absorption toward secretion (Fig. 2), a change that has been related to the permeabilizing effect of Zot on the paracellular pathway in vivo (10). This effect was detectable as soon as 20 min after Zot perfusion in the small intestine, and was completely reversible within 60 min of its withdrawal (Fig. 2). Zot also reversibly increased the serum concentration of both ^{125}I-insulin and the non-absorbable marker ^{14}C-polyethylene glycol (PEG)-4000 from the jejunum and the ileum, but not from the colon (Fig. 3). Similar results were obtained with IgG, whereby Zot (1.1×10^{-10} M) induced twofold and sixfold increases of ^{125}I-IgG absorption in the jejunum and ileum, respectively (Fig. 4). Again, no increases in absorption were detected in the colon (Fig. 4). Zot also increased the concentration of both ^{125}I-IgG and ^{14}C-PEG-4000 in the efferent mesenteric vein of the perfused small intestinal segments (data not shown).

Oral delivery of insulin in BB/Wor diabetic rats

Acute treatment. We subsequently evaluated the bioactivity of insulin after enteral co-administration with Zot to acute type 1 diabetic male BB/Wor rats (15, 16). Insulin was orally administered to animals with or without Zot, and the blood glucose levels of the rats were serially measured. After oral administration of insulin alone (given at doses between 5 and 30 IU), blood glucose levels of treated animals were not appreciably lowered (Table I). In contrast, when insulin at doses as low as 10 IU was orally coadministered with Zot 1.1×10^{-10} mol (5 μg), a significant reduction in blood glucose concentration was observed (Table I and Fig. 5). This decrement was comparable to that seen with a conventional dose of SQ insulin (Ta-

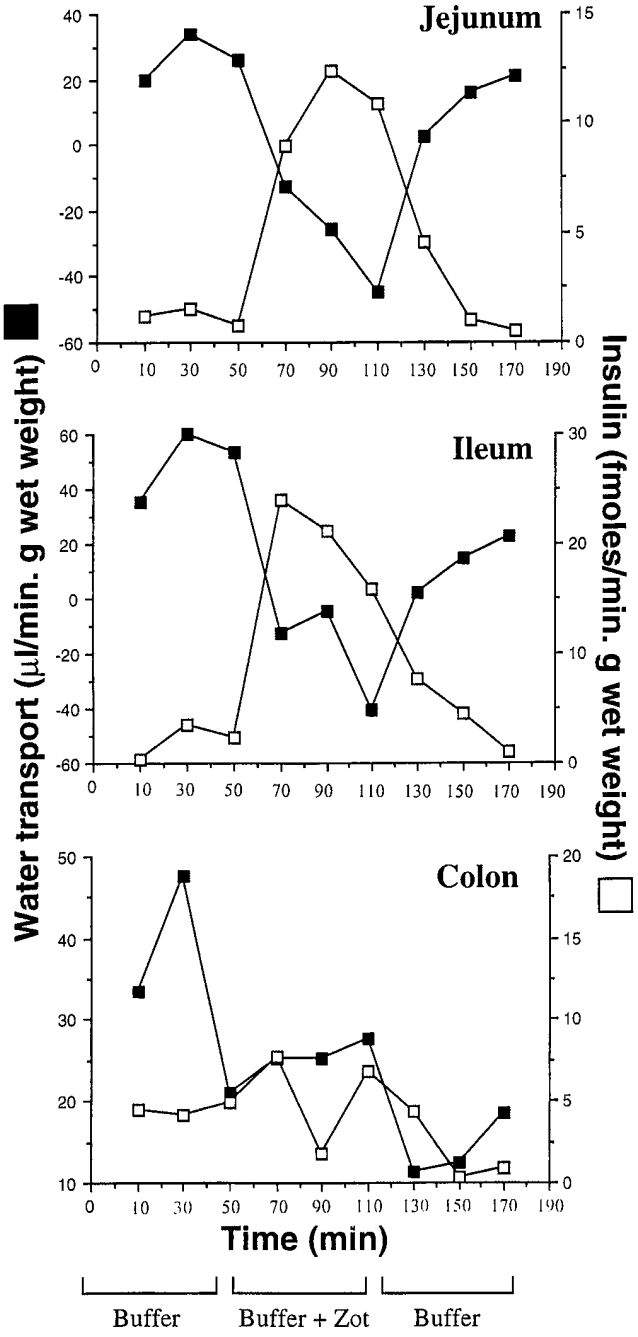

Figure 2. Effect of purified Zot on water (■) and insulin (□) transport, as determined by the in vivo perfusion assay, in rabbit jejunum, distal ileum, and colon. Note the reversible increment of insulin absorption that Zot induced in the small, but not in the large, intestine. This effect coincided with the decreased absorption of water evoked by the toxin. A representative experiment is shown.

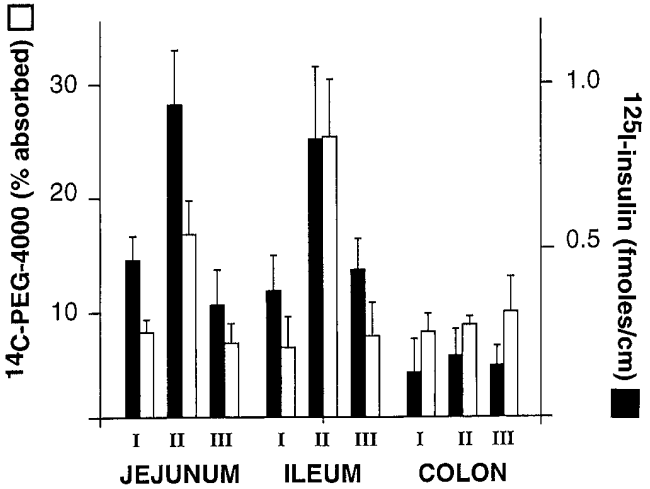

Figure 3. Serum concentrations of ^{125}I-insulin (■) and ^{14}C-PEG-4000 (□) in the presence (*period II*) or absence (*periods I and III*) of Zot in the perfusion solution. At the end of each period (see methods section), the efferent mesenteric vein of the perfused segment was cannulated to measure the amount of ^{125}I-insulin and ^{14}C-PEG-4000 that reached the bloodstream. Zot reversibly increased the serum concentration of insulin following the permeabilization of the small intestine, as suggested by the increased passage of PEG-4000 in the bloodstream. No changes were observed in the colon.

ble I), and returned to baseline by 6 h post-administration (Fig. 5). There was a significant difference in mean glucose level between the oral insulin treatment and the oral insulin + Zot treatment ($P = 0.002$), as well as a significant time x group interaction ($P = 0.03$), reflecting the drop in glucose level in the presence of Zot. By *t* tests, the difference in glucose level reached statistical significance between 60 and 120 min post administration (Fig. 5). Administration of Zot alone did not alter the blood glucose levels (Table I and Fig. 5). Mean (±SE) time to reach the blood glucose nadir after oral treatment with insulin + Zot (97±12 min) was not different from that observed in the rats receiving parenteral insulin (90±19 min). Furthermore, increased amounts of either Zot (up to 4.4×10^{-10} mol) or insulin (up to 30 IU) each induced a dose-dependent decrement of blood glucose (Table I). No toxicity was observed in the treated animals.

Prolonged treatment. To establish whether repeated Zot administration had any adverse effect on the survival of instrumented animals, survival times were compared in diabetic rats daily treated with oral insulin 10 IU, with or without 1.1×10^{-10} mol Zot. After the administration of insulin + Zot, postoperative survival time (average 84 h) and decrease in blood glucose levels 60 min after treatment (average 31%) were comparable to those observed in parenterally treated animals (96 h survival and 44% blood glucose decrement, respectively). In contrast, rats treated with oral insulin alone (10 IU) survived for only 60 h after jugular vein cannulation, and their blood glucose levels increased by 19% 60 min after treatment. None of the animals treated with insulin + Zot experienced diarrhea, fever, or other systemic symptoms, and no structural changes could be demonstrated in the small intestine on histological examination (data not shown).

Discussion

The intestinal epithelium represents the largest interface (more then 2,000,000 cm^2) between the external environment and the internal host milieu, and constitutes the major barrier through which molecules either can be absorbed or secreted. The paracellular route is the dominant pathway for passive transepithelial solute flow in the small intestine, and its permeability de-

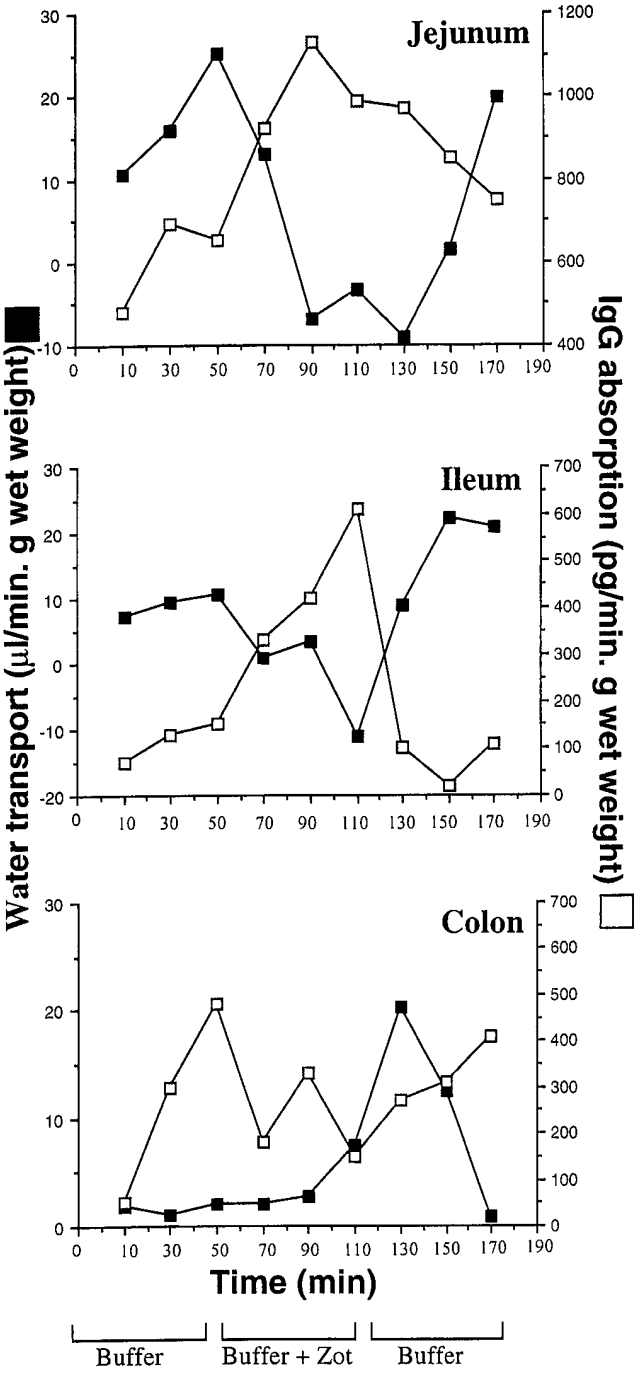

Figure 4. Effect of purified Zot on water (■) and IgG (□) transport as determined by the in vivo perfusion assay in rabbit jejunum, distal ileum, and colon. The experiment was carried out as described in the legend to Fig. 2. In this set of experiments the insulin in the perfusion solution was substituted with ^{125}I-IgG 8.33 ng/ml.

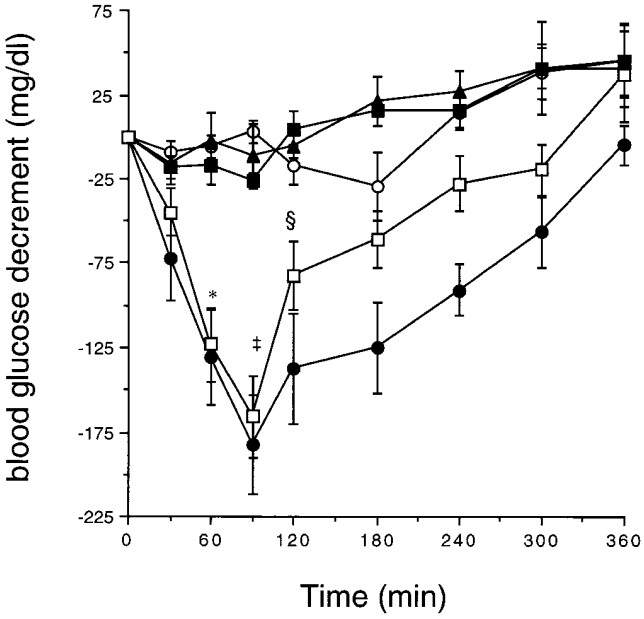

Figure 5. Effect of oral insulin 10 IU, alone (■) or in the presence of Zot 5 µg (□) on serum glucose decrement in BB/Wor diabetic rats. The coadministration of Zot induced a reduction in blood glucose concentration comparable to that seen with a conventional dose of SQ insulin (●), and returned to baseline by 6 h postadministration. Blood glucose decrement of untreated animals (○) and animals treated with oral Zot alone (▲) are shown for comparison. $n = 3$. $^{*}P = 0.005$; $^{‡}P = 0.003$; $^{§}P = 0.009$, as compared to oral insulin alone.

pends on the regulation of intercellular tj (17). A century ago, these structures were thought to be a secreted extracellular cement forming an absolute and unregulated barrier within the paracellular space (18). Physiological studies of the past several decades have shown that the tj is a dynamic structure whose physiological regulation remains largely undefined (17).

In the past few years, we have witnessed an explosion in research aimed at creating new drug delivery systems (19). Unfortunately, attempts so far to find ways to increase paracellular transport by loosening intestinal tj have been hampered by unacceptable side effects induced by the potential absorption enhancing agents. (3–6). For the most part, these agents fall within two classes: (*a*) calcium chelators, and (*b*) surfactants (5). Both types have properties which limit their general utility as a means to promote absorption of various molecules. In the case of calcium chelators, Ca^{2+} depletion induces global changes in the cells, including disruption of actin filaments, disruption of adherent junctions, and diminished cell adhesion (6). In the case of surfactants, the potential lytic nature of these agents may cause exfoliation of the intestinal epithelium, irreversibly compromising its barrier functions (5).

Considering these limitations, it was worth examining whether our previous findings on Zot modulation of tj permeability could be applied to develop alternative approaches to enhancing drug absorption through the paracellular route. We have recently demonstrated that Zot activates a complex intracellular cascade of events that regulate intestinal permeability (9). Zot induces a dose- and time-dependent PKCα-related polymerization of actin filaments strategically localized to regulate the paracellular pathway (9). These changes are a prerequisite to opening of tj, and are evident at a toxin concentration as low as 1.1×10^{-13} M (10). The toxin exerts its effect by interacting with a specific surface receptor that is present in the small intestine, but not in the colon (10). Our previous data suggest that the regional distribution of Zot receptors coincides with the different permeabilizating effect of the toxin on the various tracts of intestine tested (10). We have also demonstrated, both in vivo (10 and present paper) and in vitro (7, 10,

Table I. Effect of Oral Insulin, Alone or Coadministered with Purified Zot on the Blood Glucose Levels of BB/Wor Diabetic Rats

Treatment	*n*	Animal weight	Baseline blood glucose	Blood glucose decreament	Time to reach nadir blood glucose decreament
		g	*mg/dl*	*mg/dl*	*min*
No treatment	3	217.0±34	235.1±41.7	29.1±20.1	180±60
Parenteral insulin (SQ) (1.2–2.4 IU)	5	237.0±17	275.6±26.7	182.4±29.3*	90±19
Oral Zot (1.1×10^{-10} mol)	4	248.7±31	248.0±43.9	26.0±12.7	75±9
Oral insulin (5 IU)	2	247.5±13	229.0±48.0	15.0±7.5	78±17
Oral insulin (5 IU) + Zot 1.1×10^{-10} mol	2	239.5±15	273.8±12.5	85.5±33.5	75±15
Oral insulin (10 IU)	3	238.0±20	249.0±9.5	26.0±4.0	110±50
Oral insulin (10 IU) + Zot 1.1×10^{-10} mol	3	223.0±22	262.0±67.1	161.0±24.6‡	93±28
Oral insulin (20 IU)	3	267.5±35	318.5±15.5	66.5±38.5	140±70
Oral insulin (20 IU) + Zot 1.1×10^{-10} mol	3	267.5±36	364.5±64.5	168.0±41.0	75±45
Oral insulin (30 IU)	6	211.2±9	244.7±66.7	70.2±33.5	116±33
Oral insulin (30 IU) + Zot 2.2×10^{-10} mol	4	247.0±12	308.0±98.0	193.5±40.5§	106±75
Oral insulin (30 IU) + Zot 4.4×10^{-10} mol	5	208.0±4	362.4±53.4	214.3±7.5‖	139±30

Oral insulin, when orally coadministered with Zot, decreases serum glucose concentration of diabetic rats to levels comparable to those obtained with the parenteral administration of the hormone. *$P = 0.01$ vs. no treatment, $P = 0.007$ vs. oral insulin (10 IU), $P = 0.003$ vs. oral Zot (1.1×10^{-1} mol); ‡$P = 0.003$ vs. oral insulin (10 IU), $P = 0.006$ vs. oral Zot (1.1×10^{-10} mol); §$P = 0.047$ vs. oral insulin (30 IU), ‖$P = 0.004$ vs. oral insulin (30 IU).

and present paper) that the effect of Zot on tissue permeability occurs within 20 min of the addition of the protein to the intestinal mucosa, and is readily reversible once the toxin is removed. The in vivo experiments in BB/wor diabetic rats presented in this study also showed a transient permeabilization effect of the toxin; the blood glucose levels of the animals orally treated with insulin + Zot decreased within 30 min of co-administration of the compounds, reached its nadir after 90 min, and returned to baseline values after 6 h (see Fig. 5).

Zot displays multiple properties that make it the most promising tool currently available to enhance drug and peptide transport through the intestinal mucosa. Zot (*a*) is not cytotoxic and does not affect the viability of the intestinal epithelium ex vivo (7, 9); (*b*) fails to completely abolish the intestinal transepithelial resistance (7, 9, and present paper), (*c*) interacts with a specific intestinal receptor whose regional distribution within the intestine varies (10), (*d*) is not effective in the large intestine where the presence of the colonic microflora could be potentially harmful if the mucosal barrier was compromised (10 and present paper), (*e*) does not induce acute systemic side-effects (for at least 80–90 h) when orally administered (present paper), and (*f*) induces a reversible increase of tissue permeability (7, 9, and present paper). Our results demonstrate that coadministration of Zot with biologically active ingredients enhances intestinal absorption of the active molecule, and that this enhancement is effective for both relatively small (insulin, 5733 D) and large molecules (IgG, 140–160 kD). Furthermore, the experiments in BB/Wor diabetic rats demonstrate that orally delivered insulin can retain its biological activity without provoking severe hypoglycemia within the range of the insulin administered (i.e., up to 15 times more than the effective parenteral insulin dose). These findings have important practical implications, since the insulin therapeutic index (i.e., the ratio between the median toxic dose and the median therapeutic dose) is relatively low.

Our data suggest that the modulation of intestinal tj may be used for the oral administration of molecules normally not absorbed through the intestine. Further studies are needed, however, to establish the possible clinical application of this system for the treatment of diseases that currently require frequent and long-life parenteral drug administration.

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