Pharmacodynamics of Insulin Following Intravenous and Enteral Administrations of Porcine-Zinc Insulin to Rats

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Previous work from this laboratory showed site-dependent variations in the apparent permeability of insulin as measured using the everted rat gut sac technique, with the greatest permeability in the distal jejunum and the lowest in the duodenum (5). To quantify better the rate and extent of insulin absorption from the small intestine, closed-loop *in situ* experiments were performed in nondiabetic rats. Results correlated with the everted gut sac technique in that the absolute bioavailability determined *in situ* was higher for insulin solution administered to the more distal region of the intestine (0.133%) than that absorbed from an earlier portion of the intestine (0.059%). While the difference in regional bioavailabilities was not significant ($P = 0.08$), the blood glucose response showed highly significant differences ($P = 0.0015$), with severe and prolonged hypoglycemia resulting from insulin delivered to the distal jejunum/proximal ileum. Insulin administered iv followed a two-compartment pharmacokinetic model. Whole-body elimination rate constants were similar for both iv and enteral insulin. Although therapeutic quantities of insulin were absorbed from the distal small intestine, absorption enhancers would be necessary to decrease the dose of insulin required.

KEY WORDS: insulin; intravenous; enteral; pharmacokinetics; bioavailability; pharmacodynamics.

INTRODUCTION

The treatment of Type I diabetic patients (and some Type II diabetics) requires subcutaneous insulin injections, normally once or twice each day. For more intensive treatment, and better metabolic control, diabetics may receive as many as four injections daily. For chronic administrations, the parenteral route is not the ideal means for insulin delivery.

Oral administration, if possible, not only will be the most acceptable from a patient compliance standpoint but also will deliver insulin to the liver via the portal vein exactly as would be observed with endogenous insulin. Therefore, oral delivery would more closely mimic natural insulin secretion from the pancreas. Studies in human, insulin-dependent diabetics who also lacked pancreatic digestive enzymes have shown that insulin delivered to the upper portion of the small intestine was absorbed in small quantities based on plasma insulin levels and/or blood glucose depression (1,2). Others have also directly shown the ability of intact, active insulin to be absorbed in small amounts from a particular area of the small intestine (3,4). We have proposed (5) that regional differences in the small intestine (6) might affect the overall extent of insulin absorption and thus could influence the choice and design of a dosage form for oral insulin delivery.

Previous work from this laboratory has discussed the site-dependent intestinal mucosal permeability of insulin (5). The regions of the small intestine with optimal permeability of insulin were the distal jejunum, followed by the ileum. Moreover, brush border metabolism of intact insulin was found to be negligible or absent. Based on these *in vitro* results, studies were performed using a more physiologically normal *in situ* procedure to evaluate better whether greater insulin absorption and blood glucose response resulted from delivery to more distal as compared to more proximal regions of the small intestine.

The present report describes in detail the pharmacokinetics and pharmacodynamics of insulin following intravenous administration of porcine-zinc insulin to rats. This report also describes the compartmental and noncompartmental approaches to the evaluation of *in situ* intestinal absorption and bioavailability of insulin and corresponding blood glucose effects. These studies are useful for examining the results of insulin delivery to different regions of the small intestine and were necessary for establishing a baseline to compare the effects of absorption enhancers on insulin absorption at a particular site (which will be described in subsequent reports).

MATERIALS AND METHODS

Materials

Crystalline porcine-zinc insulin (Lot No. 009HC7; potency, 28 U/mg) was kindly donated by Eli Lilly and Company (Indianapolis, IN). The buffer solutions were prepared with analytical reagent-grade chemicals. A sterile 0.9% sodium chloride solution for intravenous use (Abbott Laboratories, North Chicago, IL) was used for the replacement of blood volume taken during sampling. Heparin solutions were prepared by diluting 1000 USP U/ml heparin sodium injection, USP (Lyphomed, Rosemont, IL), with saline to a final concentration of 10 U/ml.

Preparation of Insulin Solution

Buffer components are known to influence the intestinal passage of water and drugs (7,8). Studies involving *in situ* intestinal absorption of theophylline recommended normal saline as the medium, however, a diluted phosphate buffer also did not significantly alter absorption (8). Since insulin needs to be dissolved initially in pH 2 hydrochloric acid for solubilization purposes, adjustment of the administering solution to neutral pH was necessary. Since pH 2 is too acidic for intestinal studies and the isoelectric point of insulin is near pH 5.5, the function of the administering solution, pH 7.4 phosphate-buffered saline (PBS), was to present insulin

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in solution composed predominantly of saline and having a neutral pH. With only a low buffer capacity, this solution was not intended or expected to maintain a given pH once in the intestinal lumen, as buffers are believed to have little, if any, effect on the micro-pH at the membrane interface (7,8) and the ability of the rat small intestine to alter the bulk pH of more concentrated buffers toward neutral pH has been observed (7). The buffered saline employed throughout the intestinal absorption experiments was thus prepared by diluting a 0.0667 *M* to 0.01 *M* phosphate. Osmolality was measured with an Osmette S Model 4002 osmometer (Precision Systems, Inc., Natick, MA) and was adjusted with sodium chloride to a final value of 225–230 mOsm/kg.

Intestinal Absorption with the Closed-Loop Technique

Male Sprague–Dawley rats weighing 175–250 g were fasted for 16–20 hr prior to an experiment. Water was allowed ad libitum. The animals were anesthetized by an intraperitoneal injection of a mixture of 90 mg/kg ketamine and 10 mg/kg xylazine. One-third to one-half of the original dose was administered every 45–60 min thereafter to maintain anesthesia/analgesia. The core body temperature was maintained close to 37°C by placing the animal on a platform above a 40°C water bath with a 100-W light bulb and a reflector above.

Cannulation of the right external jugular vein was performed by inserting a 3-in. piece of Silastic tubing, 0.047-in. O.D. (Dow Corning, Midland, MI). A collar made from a 1-cm piece of PE 200 polyethylene tubing (Becton Dickinson, Parsippany, NJ) was attached to the outer end of the Silastic tubing. Before insertion, the cannula was filled with saline containing 10 U/ml heparin. Microdissecting scissors were used to cut a small opening in the jugular vein, and one tip of a microdissecting forceps, extra delicate, was inserted through the hole to guide the insertion of the cannula toward the heart. Surgical thread underneath the vein was tied around the collar of the cannula to secure it. A 23-gauge needle, filed at the end to smooth out the bevel, was inserted into the cannula and was used with a heparinized 1-ml plastic syringe for the removal of samples.

Next a midabdominal incision was made to expose the small intestine. Intestinal segments were measured with a string to a 15-cm length. Experiments were carried out in individual animals to test for insulin absorption from the distal duodenum/proximal jejunum (beginning 3–4 cm beyond the ligament of Trietz; $n = 6$) or from the distal jejunum/proximal ileum (beginning 16 cm above the cecum; $n = 6$). The desired segment was opened at each end and a piece of Tygon tubing, 4-mm o.d., was inserted into the proximal opening. A peristaltic pump (Model 1203, Harvard Apparatus, Millis, MA) was employed to perfuse normal saline through a warming chamber into the intestine to remove any residual gut contents. A total of 30 ml saline was pumped at a rate of 3 ml/min. Each segment was carefully ligated both above and below the incisions to prevent any fluid loss. Air was then pumped through the segment to remove any residual saline. The distal end of the segment was then ligated and the appropriate solution (approximately 0.6 ml) was instilled. The concentration of insulin solutions employed was 4.5 mg/ml, or 126 U/ml, while control experiments utilized an equivalent volume of blank PBS. Finally, the proximal end of the intestinal segment was quickly ligated to form a closed sac, which was carefully returned to its original place inside the peritoneal cavity.

Intravenous Administration

In order to determine the absolute bioavailability of enterally absorbed insulin, intravenous administration was necessary. Insulin (0.25 U/ml) in 0.01 *M* phosphate-buffered saline was injected at a dose of 0.2 U/kg into the lateral tail vein of fasted, cannulated nondiabetic rats ($n = 4$). Blood samples (210–230 μl) were obtained at 0, 1, 4, 7, 11, 15, 20, 30, 45, 60, 90, 120, 180, and 240 min postadministration to determine both blood glucose levels and plasma insulin concentrations. Plasma was separated by first collecting the blood samples into heparinized Natelson capillary tubes kept on ice and then centrifuging in a Damon CRU-5000 refrigerated centrifuge (IEC, Needham Heights, MA) at 3000*g* for 15 min. Plasma samples were quickly frozen in dry ice–acetone and then stored at −20°C until analysis. Blood sample volumes were replaced with the same volume of warm (37°C) saline.

Analytical Methods

Blood Glucose

Blood samples were immediately tested for glucose levels using Chemstrip bG reagent test strips (Boehringer Mannheim Diagnostics, Indianapolis, IN) with an AccuCheck II blood glucose monitor (Boehringer Mannheim Diagnostics). The sample size was approximately 30 μl of whole blood. The precision of the assay was found to be within ±3% ($n = 5$) and measurable glucose levels ranged from 10 to 500 mg/dl.

Plasma Insulin

Plasma insulin levels were quantitated using Coat-A-Count insulin radioimmunoassay kits (Diagnostics Products Corporation, Los Angeles, CA). The method utilized a solid phase radioimmunoassay technique where a standard amount of ^{125}I-labeled insulin competes with unlabeled sample insulin for binding to insulin-specific antibodies immobilized to the wall of a polypropylene tube. This technique can be used to detect as little as 1 μIU/ml insulin and concentrated samples can accurately be measured upon dilution with blank serum provided with the kit.

Frozen plasma samples were thawed at room temperature and then aliquots were pipetted into the insulin-antibody coated tubes and diluted with blank serum as needed to generate a final sample volume of 120 μl. Next 600 μl ^{125}I-labeled insulin was added, followed by vortexing for 30 sec. Duplicate standards were run to generate a standard curve (each time insulin samples were analyzed) by adding 120 μl of insulin standards with 600 μl of ^{125}I-labeled insulin. Following incubation at room temperature for approximately 18 hr, the tubes were decanted and the contents counted for 1 min with a Beckman Model 5500 gamma counter (Fullerton, CA).

Pharmacokinetic Data Analysis

The areas under both the plasma insulin– and the blood glucose–time curves were calculated by the linear trapezoidal method. The areas under the plasma insulin concentration–time curves (AUC) above controls were calculated up to 90 min following insulin administration or until the plasma insulin levels fell below the control levels. If the insulin levels at 90 min were still higher than in control experiments (16 μU/ml), the last three points were extrapolated to a time point where plasma insulin concentration reached 16 μU/ml and the AUC of this region was also included.

Since intravenously administered insulin appeared to follow a two-compartment model, the whole-body elimination rate constant was estimated for each route of administration by linear regression of semilog plots of plasma insulin versus time. The slopes of these plots between approximately 8 and 20 min were utilized to calculate the whole-body elimination rate constant. In addition, a terminal elimination rate was determined from the slope between 30 and 60 min. Apparent absorption rate constants have questionable significance due to the extremely low amount of insulin absorbed and, thus, were not included.

Statistical moment-derived noncompartmental pharmacokinetic parameters were also determined. The areas under the first moment curve (AUMC) were calculated and then the mean residence times (MRT) of insulin in the body following intravenous and enteral administrations were evaluated from Eq. (1).

$$\text{MRT} = \text{AUMC/AUC} \tag{1}$$

From MRT calculations of insulin following intravenous and enteral administrations, the mean absorption times associated with insulin intestinal absorption were obtained according to Eq. (2).

$$\text{MAT}_{\text{intestinal}} = \text{MRT}_{\text{intestinal}} - \text{MRT}_{\text{iv}} \tag{2}$$

Pharmacodynamic Data Analysis

Blood glucose depression due to insulin absorption was analyzed in three different manners. First the areas under the glucose depression–time curve (AUC_G) between the control values and the experimental values were calculated, with higher AUC_G values indicating greater blood glucose depression. The maximum blood glucose depression (minimum glucose level) was chosen as the second parameter for pharmacodynamic evaluation. Finally, the duration over which blood glucose levels remained below 70 mg/dl was determined for each experiment. The value of 70 mg/dl was arbitrarily chosen as a marker distinguishing normoglycemia and mild hypoglycemia. In cases where prolonged hypoglycemia persisted, extrapolation of the last glucose level to normoglycemia was not performed because of uncertainty about the glucose response resulting from the sustained surgical anesthesia and hypoglycemia.

RESULTS

Studies were originally begun with streptozotocin-induced diabetic rats manifesting low plasma insulin and elevated blood glucose levels. While similar absorption experiments showed increased plasma insulin and corresponding blood glucose depression, the high variability of initial blood glucose levels was unsatisfactory, as this could variably affect systemic insulin uptake and metabolism. For this reason, all experiments reported were performed using nondiabetic rats.

Intravenous administration of insulin (0.2 U/kg) resulted in plasma concentration–time profiles characteristic of an apparent two-compartment pharmacokinetic model. The insulin concentrations returned to basal levels within 30 min. The concentrations above basal level generated the biexponential Eq. (3), where preexponential and exponential terms have the units of μU/ml and min^{-1}, respectively.

$$C = 1735.78\, e^{-0.84t} + 65.97\, e^{-0.06t} \tag{3}$$

As depicted in Fig. 1, the line generated by Eq. (3) did fit the experimental data. The distribution half-life ($t_{1/2\alpha}$) of insulin was only 0.825 min, while the overall elimination half-life ($t_{1/2\beta}$) was found to be 11.45 min.

The compartmental micro-rate constants (k_{10}, k_{12}, and k_{21}) were then determined. The constant k_{10} refers to the first-order elimination rate constant from the central compartment, whereas k_{12} and k_{21}, respectively, denote first-order intercompartmental transfer rate constants from the central to the peripheral compartment, and vice versa. The values of k_{10}, k_{12}, and k_{21} are 0.571, 0.120, and 0.089 min^{-1}, respectively. The volume of distribution of insulin in the central compartment (V_c) was calculated to be 111.0 ml/kg, whereas the steady-state volume of distribution, calculated as $V_c + (k_{12}V_c)/k_{21}$, was found to be 260.11 ml/kg.

Insulin administered *in situ* to the small intestine using the closed-loop technique resulted in peak levels that were significantly different from control experiments. Instillation of 400 IU/kg insulin to the duodenum/proximal jejunum segment generated an average plasma insulin peak of 93.3 μU/ml, while the same dose administered to the more distal portion of the intestine, the distal jejunum/early ileum, produced an average plasma insulin peak concentration of 169.8 μIU/ml (Table I). As illustrated in Fig. 2, insulin profiles generated as a result of 400 IU/kg administered to the more distal region (distal jejunum/proximal ileum) showed a trend for higher absorption than for insulin administered to the more proximal region, however, the corresponding C_{peak}'s were not significantly different.

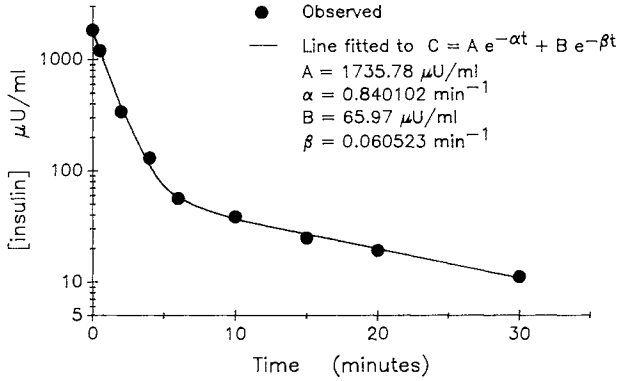

Fig. 1. Fit of the observed plasma insulin concentrations to the equation for a two-compartment kinetic model.

Table I. Insulin Bioavailability and Peak Concentrations Following *in Situ* Administration of 400 U/kg Insulin to the Small Intestine

Location	Insulin bioavailability (%)[a]	C_{peak} (μIU/ml)[a]	t_{peak} (min)
Duodenum/jejunum (n = 6)	0.059 (0.019)	93.3 (23.2)	8
Jejunum/ileum (n = 6)	0.133 (0.046)	169.8 (76.2)	8

[a] Mean (±SE).

While there was more than a twofold difference in the AUCs between duodenum/proximal jejunum and lower jejunum/early ileum administrations, this was not statistically significant (one tailed t test, P = 0.08) due to the fairly large intersubject variation in plasma data as is common with poorly absorbed drugs. As summarized in Table I, the absolute bioavailability of insulin following intestinal administration to the proximal segment was only 0.059%, which increased to 0.133% when insulin was delivered to the distal portion of the intestine. The general trend of insulin absorption was consistent with our previously reported *in vitro* everted gut sac results, which showed that the apparent mucosal permeability of insulin was higher in the distal segment of the intestine (distal jejunum/proximal ileum) (5), yet overall absorption of insulin was very low and variable, as was to be expected.

The hypothesis for site-dependent insulin absorption becomes more credible when we examine the pharmacodynamic results. Figure 3 clearly demonstrates that a significantly greater hypoglycemic response was produced by insulin administered to the distal jejunum/proximal ileum segment (one tailed t test, P = 0.0015) as compared to the duodenum/proximal jejunum segment. The blood glucose levels returned to normal values within 4 hr or less when insulin was delivered to the proximal intestinal segment, however, significant hypoglycemia persisted at least for the duration of the experiment when the hormone was administered to the distal section. Blood glucose responses have been summarized in Table II. The AUC reflecting blood glucose depression following administration to the distal jejunum/proximal ileum segment was approximately twice that of the AUC generated from the duodenum/proximal jejunum segment. Similarly, the AUCs from the plasma insulin concentration–time profiles also reflected a ratio of close to two. Furthermore, the minimum blood glucose level resulting from insulin administration to the distal portion of the intestine was significantly hypoglycemic, whereas insulin delivered to the proximal portion of the intestine resulted in only mild glucose depression, with levels within the limits of normoglycemia. While intravenous administration of insulin to the peripheral circulation caused glucose levels to drop below 70 mg/dl for an average of 30 min, insulin delivered to the distal jejunum/early ileum segment produced persistent hypoglycemia.

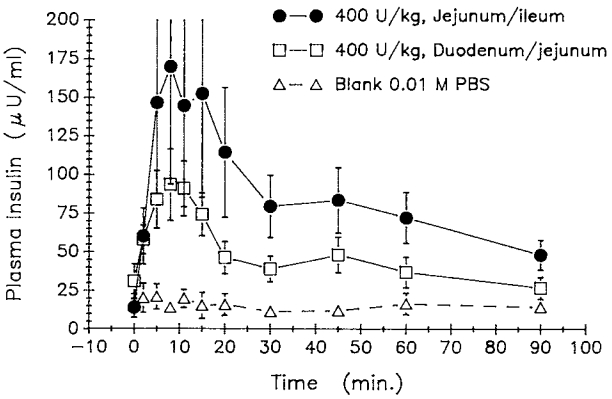

Fig. 2. Plasma insulin levels (mean ± SE) after *in situ* closed-loop administration of 400 U/kg insulin to the distal jejunum/proximal ileum (n = 6) and to the distal duodenum/proximal jejunum (n = 6) or blank 0.01 M phosphate-buffered saline administered to the distal jejunum/proximal ileum (n = 5).

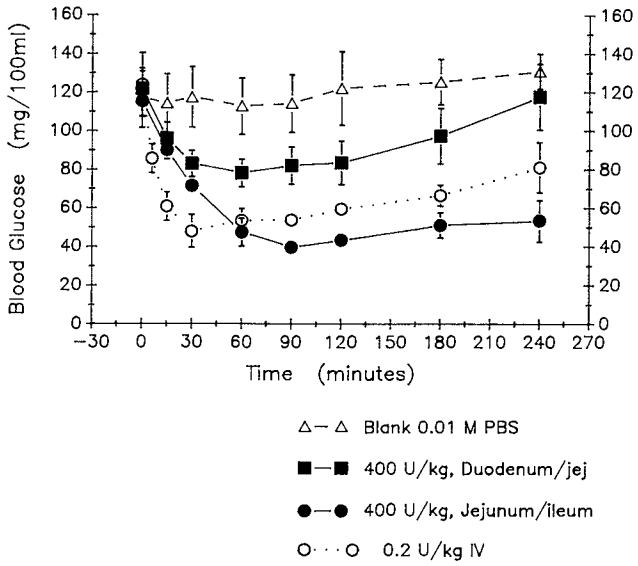

Fig. 3. Blood glucose levels (mean ± SE) after *in situ* closed-loop administration of insulin to the distal jejunum/proximal ileum (n = 6), to the distal duodenum/proximal jejunum (n = 6), or intravenously (n = 4) and blank 0.01 M phosphate-buffered saline administered to the distal jejunum/proximal ileum (n = 5).

The plasma elimination rate constants were found to be very similar for insulin absorbed from both duodenum/proximal jejunum and distal jejunum/ileum segments as summarized in Table III. The elimination phase is followed by a

Table II. Blood Glucose (bG) Response Following Administration of Insulin Intravenously or *in Situ* to the Small Intestine (Mean ± SE)

Conditions	bG AUC (mg min/dl)	bG_{min} (mg/dl)	Duration bG <70 mg/dl (min)
IV, 0.2 U/kg (n = 4)	13,483 (671)	48.8 (6.79)	30
400 U/kg *in situ* administered to			
Duodenum/jejunum (n = 6)	7,742 (1767)	72.8 (7.10)	0
Jejunum/ileum (n = 6)	15,917 (1155)	36.5 (4.41)	>208

Table III. Elimination Rate Constants and Half-Lives Determined from *in Situ* Administration of 400 U/kg Insulin to the Distal Duodenum/Proximal Jejunum or the Distal Jejunum/Proximal Ileum

Location	Elimination rate constant and half-life			
	β (min^{-1})	$t_{1/2}$ (min)	Terminal (min^{-1})	$t_{1/2}$ (min)
Duodenum/ jejunum	0.05934	11.68	0.01099	63.05
Jejunum/ ileum	0.05977	11.60	0.01758	39.42

long shallow terminal phase probably due to redistribution of cellular insulin. The noncompartmental MRTs and MATs from enteral administrations are reported in Table IV along with the MRT from intravenous administration. The MATs from both segments were very close and were substantially greater than the MRT following intravenous administration, indicating a prolonged residence time associated with the absorption phase of the peptide.

DISCUSSION

The pharmacokinetics of intravenously administered insulin were consistent with results reported by others in that it exhibited apparent two-compartment model kinetics, it had a fairly large volume of distribution and a short half-life (10–12). Some of these studies have also examined the curve fitting of data to a three-compartment kinetic model or have included calculations from noncompartmental analysis. For the parameters reported in this study, comparisons between values calculated using compartmental and those calculated using noncompartmental approaches showed virtually the same results.

Care must be taken when comparing the pharmacokinetic parameters of insulin reported by various research groups since variations occur due to differences in dose levels, species, initial blood glucose levels, anesthesia, and whether labeled or unlabeled insulin was employed (10,13,14). For the work presented here, the AUC from basal levels of insulin as determined by control experiments was excluded from all measurements so that direct comparisons could be made for the amount of insulin actually absorbed. This decrease from the total AUC may produce a subtle effect on parameters such as volume of distribution or total-body clearance, yet these parameters were all of the same order of magnitude as in other reports. Moreover, basal levels of insulin were fairly low in the control experiments, possibly influenced by the use of xylazine as the anesthetic, which was administered every 45–60 min throughout the experiments. Xylazine, a veterinary analgesic, has been reported to induce hypoinsulinemia for a period of approximately 2 hr in rats (13).

Table IV. Noncompartmental Pharmacokinetic Parameters for Insulin Absorption

Conditions	MAT (min)	MRT (min)
0.2 U/kg, iv	—	3.02
400 U/kg, duodenum/jejunum	51.87	54.89
400 U/kg, jejunum/ileum	52.87	55.89

As calculated from the intravenous data, insulin showed a volume of distribution of 111.0 ml/kg for the central compartment. The fact that this is greater than the whole blood volume of a rat [approximately 64.1 ml/kg (15)] probably results from the uptake of insulin by receptors and its distribution to extracellular fluid (12) and lymph (16). The steady-state volume of distribution, V_{ss}, was calculated to be 260.11 ml/kg and was greater than V_c, probably as a result of extensive tissue distribution. The MRT, a noncompartmental parameter, for insulin injected into the tail vein was calculated to be only 3.02 min, and the half-life calculated from the elimination rate constant (β) ranged from only 7 to 12 min in individual experiments. A relatively rapid clearance and short half-life are not uncommon for bioactive proteins (17), resulting from both receptor uptake in the liver and other peripheral tissues and natural degradation processes.

Consistent with our previous report on insulin transport in everted gut sac experiments (5), *in situ* administration of insulin to a closed loop of the rat small intestine showed a trend for greater insulin absorption from the more distal regions of the small intestine than in the proximal regions (Table I), although differences were not statistically significant. In contrast, highly significant differences in blood glucose responses were noted, where a much higher pharmacodynamic response was observed for insulin delivered to the distal portions of the small intestine (Fig. 3). Others have demonstrated that high concentrations of insulin presented to the small intestinal mucosa resulted in absorption of small amounts of biologically active insulin as measured directly in portal venous effluent (4). Likewise, insulin immunoreactivity following administration of relatively low insulin concentrations was detected in the mesenteric vein by Ziv *et al.* (3), along with evidence of cross-reactive metabolites. While it is thus believed that very small quantities of insulin were absorbed in the experiments herein as measured systemically (preceded by hepatic uptake), the blood glucose response provided a more sensitive measurement of the absorption of active insulin. Additional studies are under way to examine directly regional differences in the appearance of insulin in the mesenteric vein of rats in similar experiments, as well as lymphatic concentrations.

The shape of the insulin plasma level versus time profiles indicates a rate of absorption being rather close in magnitude to the rate of elimination. Also, following an initial phase of elimination equal to the rate constant β determined from intravenous bolus experiments, there was a slower rate of elimination as insulin concentration returned to normal levels. In addition to distribution to tissues, these characteristics may indicate that a portion of the insulin might have been absorbed into the lymphatics, which would thus prolong absorption and overall elimination. The relatively small size of insulin and its hydrophilicity favor absorption into the blood, however, it would also be capable of entering the lymph (17). Such lymphatic absorption may also influence the MAT and MRT for insulin (Table IV), where the large MAT and only slightly higher MRT reflect a prolonged pe-

riod of absorption for the appearance of insulin in the plasma.

If differences in the regional absorption of insulin exist, the mechanistic explanation is yet to be resolved. While some studies have reported that the permeability of the small intestine decreases progressively from the duodenum to the terminal ileum (18,19), this may not always be the case when dealing with peptides and proteins. In experiments with everted gut sacs, insulin (5) and 1-desamino-8-D-arginine vasopressin (dDAVP) (20) both showed higher permeability through the ileum than the duodenum, and dDAVP absorption *in vivo* also reflected better absorption from the more distal regions of the small intestine (20). A still larger protein molecule, BSA, did not show regional differences in absorption (20). Poor correlation between absorption of relatively large protein molecules (25–68 kD) and their molecular weights has been reported (21). Thus, in addition to size, the physical and chemical properties of a specific protein are expected to affect the extent of its absorption. Therefore, the absorption insulin in a specific intestinal region would depend on the combination of the character of the intestinal biomembrane, the size and distribution of tight junctions, the extent of intracellular degradation for insulin which diffuses or is actively transported transcellularly, and the presentation and uptake into the venous or lymphatic vessels of the submucosa.

The use of nondiabetic rats in these experiments required higher insulin doses than would be required for diabetic animals. This results from extensive feedback mechanisms to maintain normoglycemia. The administration of insulin to diabetic rats with hyperglycemia would therefore show relatively greater responses to similar amounts of insulin absorbed. Nevertheless, blood glucose responses in the nondiabetic animals did show a significantly greater effect for insulin administered to the distal jejunum/proximal ileum than for that administered to the distal duodenum/proximal jejunum. The hypoglycemic effect following insulin absorption from the jejunum/ileum persisted for more than 4 hr (Fig. 3), which would be therapeutically significant. The blood glucose depression from intestinally delivered insulin, however, shows much less response when normalized for dose compared to that from intravenously administered insulin. First, it must be remembered that insulin dose and glucose response curves are not linear (22–24). Second, insulin absorbed from the intestine enters the portal vein, and in normoglycemia approximately 40% of insulin is extracted by the liver on the first pass (25). The action of insulin on the liver (suppression of hepatic glucose production) is more sensitive than the peripheral action of insulin (glucose disappearance), yet the delivery of insulin first to the peripheral circulation would allow for greater blood glucose depression than for insulin transported first to the liver (26). Therefore direct comparisons of blood glucose response following intravenous or intestinally administered insulin are difficult.

The greater blood glucose depression from insulin delivered to the distal jejunum/proximal ileum as well as the somewhat greater bioavailability determined from insulin's AUC would favor this site for targeting the release and absorption of insulin from an oral delivery system. Such a device would protect insulin not only from the acidic environment of the stomach, but also from the high concentrations of luminal enzymes in the upper small intestine. Protection from pancreatic enzymes, particularly α-chymotrypsin, is essential (27). While oral insulin might only supplement rather than totally replace injections, the availability of an oral dosage form of insulin would provide a more convenient method of administering an intensive insulin treatment regimen. By providing more insulin doses per day, this therapy more closely mimics the pancreatic output of insulin than when only one or two injections are given daily. Consequently, better control of glucose levels may perhaps decrease subsequent long-term complications (28,29). Furthermore, intestinally absorbed insulin would provide a better, more natural, balance of insulin delivered to the liver and to the peripheral tissues than present-day peripherally administered injections. Realistically, the low bioavailability observed in these studies would require the use of an appropriate absorption enhancer to provide a suitable amount of insulin to be absorbed.

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