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THE EFFECTS OF FATTY ACIDS AND THEIR DERIVATIVES ON THE INTESTINAL ABSORPTION OF INSULIN IN RAT

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Insulin solution (25 U/ml/kg, pH 8.0) was administered with 50 mM of various C8-C18 fatty acid and derivative absorption enhancers to the duodenum, colon and rectum of fasted normal rats under sodium pentobarbital anesthesia. Solutions were administered directly into ligated or sealed intestinal segments. Blood was sampled periodically from the jugular vein and blood glucose levels determined. The maximum decrease inblood glucose level and area under the depression curve were used to estimate bioavailability. Enhancing effects rectally were further investigated in streptozotocin-induced diabetic rats in both the fasted and non-fasted states. Blood glucose levels and serum insulin concentrations were determined.

INTRODUCTION

Non-parental dosage forms are desirable for insulin administration because of the inconvenience and discomfort of daily injections. Many attempts have been made to develop the possible enteral formulations. Insulin has been administered jejunally,[1] rectally,[2] and nasally.[3] Since insulin permeability is very low through epithelial cells, absorption enhancers were used. A number

of compounds have been reported to have the ability to enhance the absorption of poorly absorbed drugs, i.e., fatty acids,[4-6] glycerides,[4,7,8] surfactant,[9] salicylates[10] and enamine.[11]

In the present investigation, fatty acids and either their monoglyceride or sucrose ester derivatives were selected as absorption enhancers to induce the intestinal absorption of insulin. These compounds were used because they are biologically degradable, most of them are permitted as food additives and are considered nontoxic. Medium chain fatty acids or monoglycerides have been previously used to enhance the intestinal absorption of poorly absorbed low molecular weight compounds.[4-6] Sodium caprate (C10) and monocaprylin (C8 monoglyceride) were used to induce the rectal absorption of high molecular weight human epidermal growth factor[12] and insulin,[13] respectively. Few reports were found concerning the enhancing effects of fatty acid sucrose esters. Our aims were to compare fatty acid derivative absorption enhancing effects and to evaluate effectiveness in both normal and diabetic rats.

MATERIALS AND METHODS

Bovine insulin, fatty acids, monoglycerides, sucrose monocaprate and streptozotocin were purchased from Sigma Chemical Co. (St. Louis, MO). Sucrose esters of lauric acid, palmitic acid and oleic acid (80 % monosubstituted ester content) were obtained from Mitsubishi Kasei (Tokyo, Japan). All other chemicals were of the highest purity available.

Absorption enhancers were either dissolved or suspended by sonication in isotonic PBS pH 8.0. The pH of the solution was readjusted to 8.0 and insulin was then dissolved. Male Sprague-Dawley rats (200-250 g) were fasted for 16 hr but allowed to drink water *ad libitum*. They were anesthetized intraperitoneally with 30mg/kg sodium pentobarbital. For rectal administration, insulin solution was infused into the anus and the anus was closed with cyanoacrylate adhesive. For duodenal or colonic administration, 8

or 6 cm, respectively, pieces of polyethylene tubing (PE 50, Becton Dickinson & Co., Parsippany, NJ) were introduced into the intestine on the day prior to insulin administration through the stomach or the cecum, respectively. The tip of the tubing was covered with 1 cm silicone rubber tubing (Silastic 602-135, Dow Corning Corp., Midland, MI) to prevent the tubing from scratching the mucosa, and the other end was sealed and placed between the skin and muscle of the abdomen. This procedure reduced surgical trauma during experimentation. On the following day, this end of the tubing was lifted and insulin solution was administered through the tubing. Diabetes was induced by intravenous injection of streptozotocin (60 mg/kg). Diabetic rats were used one week after injection.

Blood samples (50 μl) were taken from the jugular vein by a direct injection. Glucose level were measured using the Accu-Check IIm blood glucose monitor (Boehringer Mannheim Diagnostics, Indianapolis, IN). For serum insulin assay, the jugular vein was cannulated with 7 cm polyethylene tubing (PE 50), the tip of which was covered with 2.5 cm silicone rubber tubing (Silastic 602-135) and 0.25 ml of blood sample was removed. The serum (100 μl) was diluted in 200 μl of 0.15 M PBS pH 7.4 containing 5 % BSA (RIA grade, Sigma, St. Louis, MO). Insulin was measured using a radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA).

RESULTS AND DISCUSSION

Generally, it is reported that absorption enhancers are more effective in the large intestine than the small intestine.[14] The enhancing effects of various fatty acids and their derivatives were compared after rectal administration. Figure 1 shows the typical blood glucose level change after rectal administration of insulin and absorption enhancer. The mean blood glucose levels at 15 and 5 min before insulin solution administration were regarded as the initial value and blood glucose levels were normalized as percent of this initial value. The enhancing effects were estimated by the

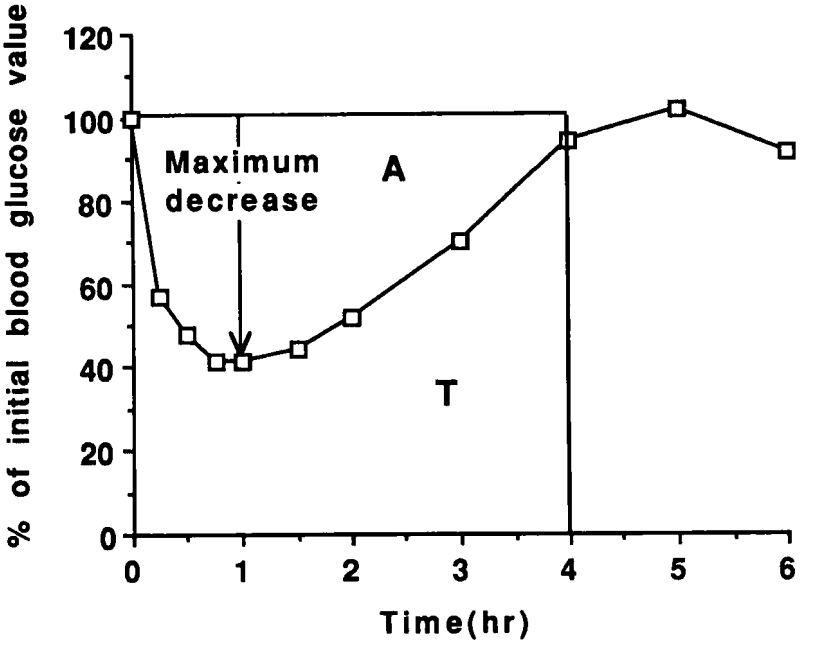

FIGURE 1
Estimation method of the enhancing effects. The % decrease of AUC is A divided by T. A is the decreased area of blood glucose level under baseline.

maximum decrease of blood glucose level and the percent decrease of area under the curve (AUC), the decreased area (A) divided by total area (T) for 4 hrs x 100 %. The percent decrease of AUC was the average decrease of blood glucose level for 4 hrs. The enhancing effects are compared in Table 1.

A critical carbon chain length was seen with fatty acids; less than 10 carbon chain fatty acids had no effect, but capric acid (C10) was the most effective enhancer. Similar critical carbon chain effects were reported for poorly absorbed low molecular weight compounds.[4-6] Lauric acid and oleic acid had smaller effects, but they themselves seem to have an ability to induce absorption when they are dissolved by a second surfactant.[4] These smaller effects were considered to be due to their low solubility.

TABLE 1
Enhancing Effects of Fatty Acids and Their Derivatives on the Rectal Absorption of Insulin in Fasted Normal Rats (Mean ± S.D., n=4)

Compound[a]	Maximum decrease of blood glucose level (% of initial value)[b]	Decrease of AUC (% over 0-4 hr)[b]
Insulin[c]	49.2 ± 5.5	22.9 ± 4.5
Buffer	16.2 ± 3.2	6.1 ± 4.5
Insulin	14.0 ± 1.9	1.6 ± 3.3
Fatty Acids		
C8:0	17.4 ± 11.7 ns	-0.2 ± 4.5 ns
C9:0	12.4 ± 6.5 ns	0.6 ± 5.2 ns
C10:0	71.0 ± 10.0 **	38.9 ± 4.9 **
C11:0	63.4 ± 11.2 **	38.8 ± 5.6 **
C12:0[d]	54.1 ± 14.1 **	16.3 ± 16.0 ns
C18:0[d]	23.2 ± 2.4 **	5.7 ± 4.9 ns
Monoglycerides		
C8:0	55.1 ± 7.3 **	26.1 ± 7.8 **
C10:0	47.3 ± 11.0 **	24.5 ± 7.6 **
C12:0[d]	15.0 ± 4.8 ns	0.3 ± 3.2 ns
C18:1[d]	19.2 ± 9.3 ns	8.5 ± 5.4 ns
Sucrose Esters		
C10:0	61.6 ± 9.0 **	39.5 ± 6.2 **
C12:0[e]	57.1 ± 6.4 **	27.8 ± 6.9 **
C16:0[d,e]	31.4 ± 16.5 ns	12.6 ± 20.7 ns
C18:1[d,e]	17.5 ± 3.9 ns	3.2 ± 1.3 ns

[a]25 U/kg insulin with 50 mM enhancer dose
[b]mean ± S.D., n=4; to insulin **: $p<0.01$, ns: not significant
[c]IV dose of 0.5 U/kg
[d]solution not clear
[e]3% concentration

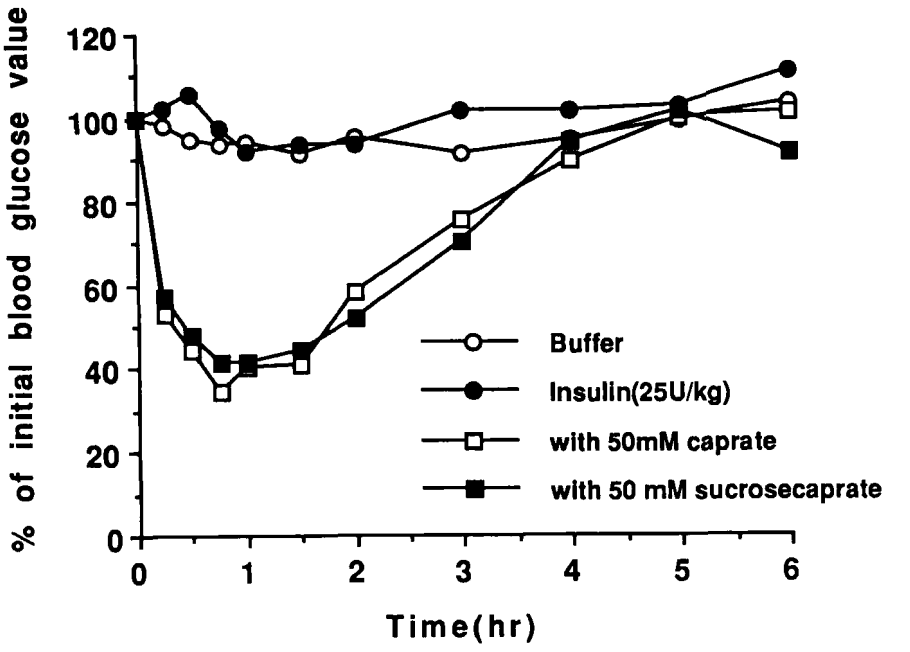

FIGURE 2
Blood glucose levels after administration to the rectum in normal rats (mean ± 13.6 % S.D., n=4).

Among monoglycerides, monocaprylin was the most effective even though caprylic acid, itself, was not effective. Sucrose caprate was as effective as sodium caprate. Other longer chain fatty acid sucrose esters were less effective than sucrose caprate. These results suggested that a certain length of hydrophobic chain (more than 9 carbons) and suitable solubility (a polar head) were needed to induce a permeability change of the mucosal membrane for insulin to be absorbed.

Since sodium caprate and sucrose caprate were the most effective enhancers, their enhancing effects were compared in the duodenum, colon and rectum. Figure 2 shows blood glucose levels after administration to the rectum. The administration of buffer solution or insulin solution alone had no effect on blood glucose levels. The coadministration of either sodium caprate or sucrose caprate with insulin caused a decrease in blood glucose levels.

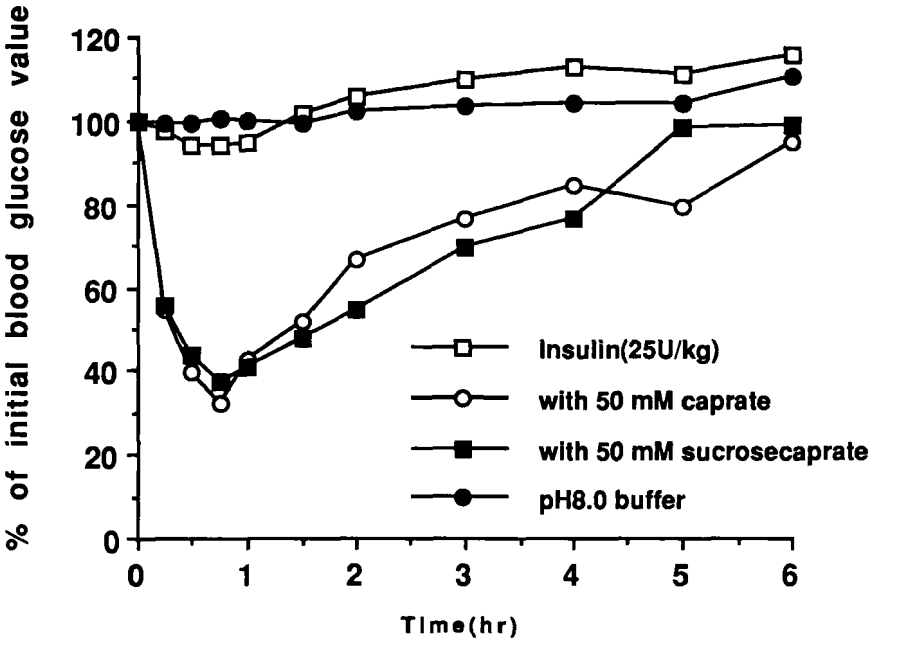

FIGURE 3
Blood glucose levels after administration to the colon in normal rats (mean ± 18.8 % S.D., n=3-4).

Maximum decreases in blood glucose levels were observed at 1 hr and blood glucose level returned to the initial value at 4 hrs. Similar blood glucose changes were observed after colonic administration (Figure 3). In the duodenum, the administration of insulin (50 U/kg) with either 250 mM sodium caprate or sucrose caprate had no effect on blood glucose levels. To see a decrease in blood glucose level, the dose of insulin was increased to 150 U/kg (Figure 4). The effects were still smaller than those seen in the colon or the rectum. When the low solubility of insulin at the actual pH (6.5) of the small intestine and the dilution of enhancers with digestive juices are taken into account, the difficulty in achieving the same effects in the small intestine is understandable.

In normal rats, insulin decreased blood glucose levels from a normal level to a lower level. In diabetes, it is important to

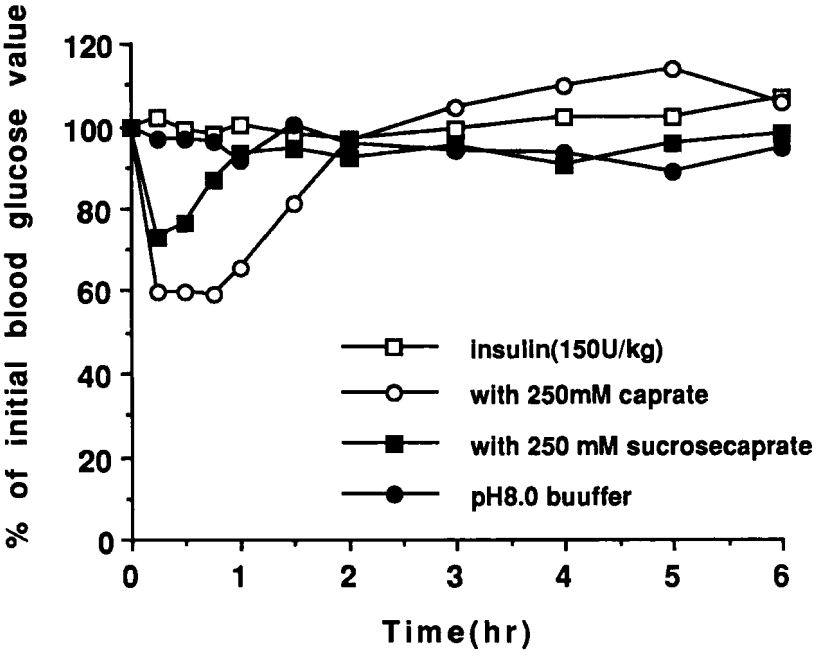

FIGURE 4
Blood glucose levels after administration to the duodenum in normal rats (mean ± 8.2 % S.D., n= 3-4).

decrease elevated levels to normal. To demonstrate effectiveness, the enhancers were evaluated in diabetic rats. Diabetes was induced by intravenous injection of streptozotocin. The average blood glucose levels were 303.2 ± 68.4 (n=24) and 402.9 ± 43.8 (n=22) mg/dl in the fasted and non-fasted states, respectively. On the other hand, the average blood glucose level of fasted normal rats was 69.7 ± 6.0 mg/dl (n=64). Figure 5 shows blood glucose levels in fasted diabetic rats. The coadministration of either sodium caprate or sucrose caprate with insulin caused a decrease in blood glucose levels. The decreasing effect was prolonged in comparison to normal rats (Figure 2). The maximum decrease in blood glucose levels was seen at 2 hrs and blood glucose levels were still lower than initial values at 6 hrs. Intravenous injection of insulin also had prolonged effects. These results suggested that the prolonged

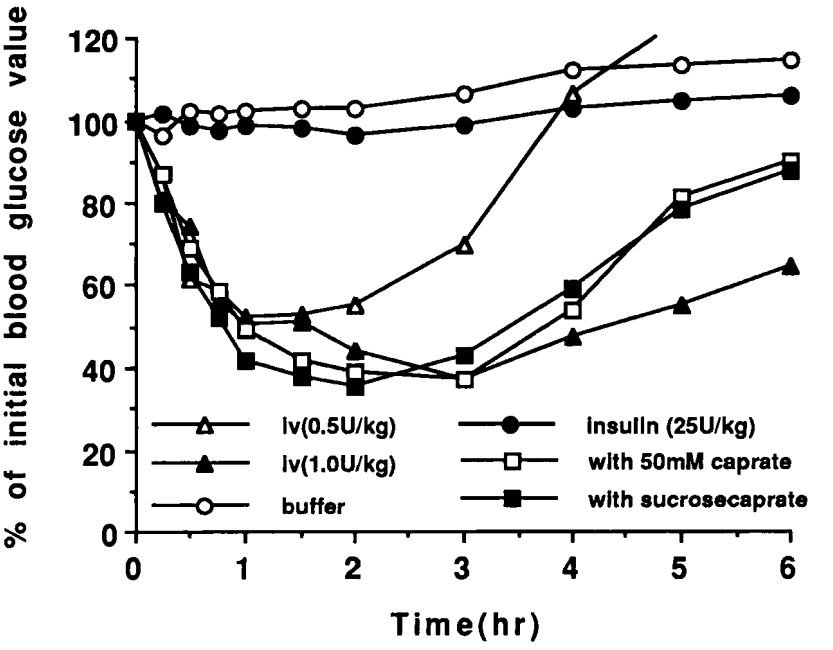

FIGURE 5
Blood glucose levels after administration to the rectum in fasted diabetic rats (mean ± 22.1 % S.D., n=4).

effects were due to a lack of a response to high blood glucose levels by diabetic rats because decreased blood glucose levels were still higher than normal levels. When compared with an IV dose (100 % absorption), 3 - 4 % of insulin was absorbed from the rectum. Blood glucose levels may be affected by many factors; to confirm the absorption of insulin, serum insulin levels were measured (Figure 6). When buffer or insulin solution alone was administered, no change in insulin levels was observed. This indicated that the experimental procedure did not induce the secretion of endogenous insulin in diabetic rats and that insulin alone was not absorbed from the rectum. An apparent increase in serum insulin levels was observed when insulin was administered with either sodium caprate or sucrose caprate. These results indicated that the decreases in blood glucose levels were due to the absorption of insulin.

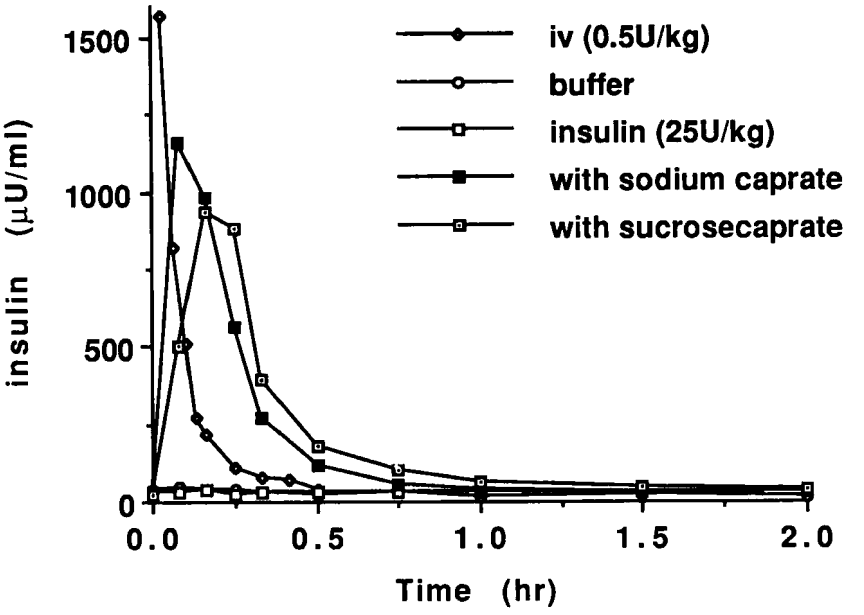

FIGURE 6
Serum concentrations of insulin after administration to the rectum in fasted diabetic rats (mean ± 35.1 % S.D., n=3-4).

Although approximately 50 % of insulin absorbed from the intestine is considered to be extracted into the liver,[15] in the case of rectal administration, we can estimate the approximate absorbed ratio using the increased AUC of serum insulin levels because most of the absorbed insulin is considered to bypass the liver and enter into the systemic circulation directly.[16] With the coadministration of sodium caprate and sucrose caprate, increased AUC of 253.1 and 245.1 μU/ml-hr indicated that 3.9 and 3.8 % of insulin were absorbed from the rectum, respectively, when compared to an IV dose (130.3 μU/ml-hr).

Because meals contribute to increased blood glucose levels, it is important to decrease blood glucose levels in non-fasted diabetic rats. Figure 7 shows blood glucose levels in non-fasted rats. The coadministration of either sodium caprate or sucrose caprate caused a decrease in blood glucose levels. The effects were smaller than

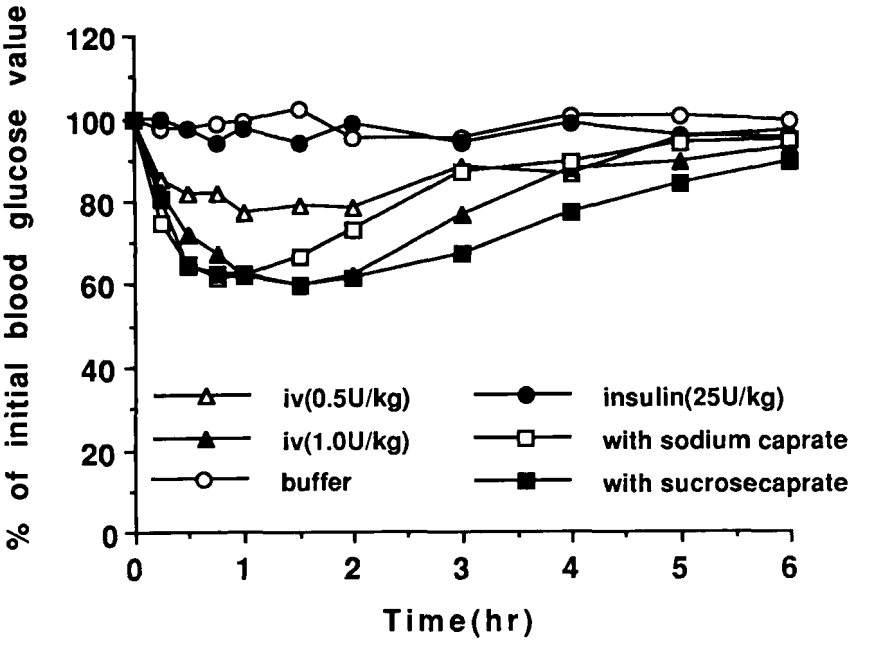

FIGURE 7
Blood glucose levels after administration to the rectum in non-fasted diabetic rats (mean ± 7.7 % S.D., n=3-4).

in fasted diabetic rats. The smaller effects was considered to be due to the intake of saccharides from the small intestine, but not due to a decrease in the absorption of insulin, because the IV dose of insulin also had a decreased effect.

The mechanism of enhancement is of great interest. Tomita and coworkers suggested that the enhancement of colonic drug absorption was through both paracellular[17] and transcellular[18] permeation routes. Even if transcellular permeability was increased for low molecular weight compounds by an absorption enhancer, it would be difficult for a high molecular weight compound to easily penetrate through the membrane while the membrane maintains its lipid bilayer structure. Capric acid increased the pore radius from 8 to 13-15 Å in the colon.[17] Bohider et al.[19] suggested that the radius of insulin was 12.7 Å surrounded by a hydration layer of 8 Å thickness. The absorption of insulin might best be induced through an enlarged paracellular route.

CONCLUSIONS

The enhancing effects of various C8-C18 fatty acids and their derivatives on insulin absorption was studied in the rectum of fasted normal rats under pentobarbital anesthesia. Bioavailability was estimated from the maximum decrease in blood glucose level and the decrease of the AUC of blood glucose level. A critical carbon chain length (C10) effect was seen with the fatty acids. Caprylic acid (C8) was not effective, but monocaprylin was effective in enhancing insulin absorption. Sodium caprate (C10) and sucrose caprate were the most effective enhancers.

Absorption was also evaluated in both the colon and the duodenum. The enhancers were effective in the colon. In the duodenum, a several fold larger dose of insulin and enhancer was required to achieve a similar level of blood glucose. This suggests that absorption in both the rectum and the colon were more sensitive to the enhancers than in the duodenum.

Enhancing effects were further evaluated in streptozotocin-induced diabetic rats. In the fasted state, the coadministration of either sodium caprate or sucrose caprate with insulin to the rectum caused a decrease in blood glucose levels. Increased serum concentrations of insulin were also seen. Bioavailability of insulin with enhancer from the rectum was estimated to be about 4 %. The decrease in blood glucose levels was also seen in non-fasted rats, but the effect was smaller than in fasted rats. The addition of these absorption enhancers to a suppository or colon targeted dosage form might induce the absorption of insulin.

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