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Research Article

Effect of Receptor Up-Regulation on Insulin Pharmacokinetics in Streptozotocin-Treated Diabetic Rats

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The present study investigated the mechanism by which the disposition of insulin is altered in streptozotocin (STZ)-treated diabetic rats as compared with 48-hr-fasted normal (control) rats. It was shown by an indocyanine green infusion method that the hepatic plasma flow rate (Q_H) in diabetic rats (1.64 ml/min/g liver) is significantly higher than that in control rats (0.982 ml/min/g liver). The portal injection technique revealed that the unidirectional clearance (CL_{on}), which represents the binding of A_{14}-^{125}I-insulin to surface receptors in the liver, is significantly elevated in diabetic rats, suggesting an increase in the surface receptor number (R_T), i.e., up-regulation in the liver. In both control and diabetic rats, the total-body clearance (CL_{tot}) and steady-state volume of distribution (Vd_{ss}) of labeled insulin decreased significantly with a simultaneous injection of unlabeled insulin (8 U/kg), confirming that the disposition of insulin is affected largely by specific, saturable receptor-mediated processes. The CL_{tot} and Vd_{ss} increased significantly in diabetic rats, while nonspecific portions of these parameters were not changed. From the increases in CL_{tot} (80%) and Q_H (67%) in diabetic rats, a pharmacokinetic analysis has revealed a 40% increase in the hepatic intrinsic clearance ($CL_{int,sp}$) of A_{14}-^{125}I-insulin via a specific mechanism in diabetic rats. In conclusion, we have provided *in vivo* evidence for a small increase in $CL_{int,sp}$ of insulin in STZ-diabetic rats compared with control rats, which may be caused by an increase in the surface receptor number in the livers of diabetic rats.

KEY WORDS: diabetic rats; streptozotocin; insulin pharmacokinetics; hepatic clearance; insulin receptor; up-regulation.

INTRODUCTION

Diabetic mellitus is a chronic disorder characterized by a raised level of glucose in the blood. Streptozotocin (STZ) selectively destroys the pancreatic β cell with production of permanent diabetes. Hypoinsulinemic diabetic animals, such as STZ-diabetic rats, have an increased binding capacity for insulin compared with control animals, due to an increased number of receptor sites on liver plasma membranes (1,2) and other target tissue membranes (3,4).

It has been recognized that an *in vivo* receptor compartment considerably affects insulin elimination and distribution (5–7). Philippe *et al.* (5) demonstrated that the metabolic clearance rate of ^{125}I-insulin was elevated in STZ-diabetic rats, and related this change to the increased binding of insulin to a specific receptor compartment. Moreover, using isolated liver perfusion experiments, Rabkin *et al.* (8) recently demonstrated that the hepatic clearance of immunoreactive insulin was significantly higher in hyperglycemic diabetic rats than that in control rats. In their study, the perfusion flow rate was set equal in control and diabetic groups. However, previous studies that compared insulin pharmacokinetics between normal and diabetic animals have disregarded a physiological change in blood flow rate, although hepatic clearance is dependent not only upon the intrinsic clearance activity but also upon the hepatic blood flow rate (9,10).

Moreover, it is known that insulin clearance is nonlinear and shows saturation at high physiologic insulin concentrations (11,12). Thus, when target tissues are exposed to different insulin concentrations in the entering blood, it is difficult to compare comprehensively the receptor number or intrinsic endocytotic activity between normal and diabetic animals in *in vivo* conditions.

Therefore, the present study examined the changes in both the hepatic blood flow rates and the portal insulin concentrations in control and STZ-diabetic rats, in order to provide a quantitative interpretation concerning the change in hepatic intrinsic clearance of insulin in a diabetic state.

MATERIALS AND METHODS

Chemicals

Human insulin ^{125}I-labeled at tyrosine-A_{14} (A_{14}-^{125}I-insulin), with a specific activity of 2000 Ci/mmol, and ^{3}H-water, with a specific activity of 5 mCi/ml, were purchased from Amersham International Ltd. (Buckinghamshire, UK). Crystalline porcine insulin and bovine serum

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albumin (BSA; Fraction V) were obtained from Sigma Chemical Co. (St. Louis, MO), streptozotocin (STZ) and trichloroacetic acid (TCA) from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and indocyanine green (ICG) from Daiichi Pharmaceutical Co. (Tokyo, Japan). All other reagents were commercially available and of analytical grade. The monoiodinated insulin was dissolved in phosphate-buffered saline (PBS) containing 0.1% BSA (designated PBS solution) and stored at −20°C until study. The labeled insulin used was at least 98% pure as assayed by both TCA precipitability and HPLC analysis. Distilled, deionized water was used throughout the experiments.

Animals

Male Wistar rats (200–230 g) were obtained from Sankyo Laboratory Co., Ltd. (Toyama, Japan) and allowed free access to standard rodent chow and water. Control rats were fasted for 48 hr before experiments, while diabetic rats were not fasted.

Induction of Diabetes

Diabetes was induced by intravenous (i.v.) injection of a freshly prepared solution of STZ (65 mg/kg in saline/0.1 *M* citrate buffer, pH 4.5). Diabetic rats were studied 2 weeks after STZ injection. Control rats were untreated. Diabetes was recognized by reduced weight gain compared with control rats and by glycosuria (without ketonuria). Plasma glucose concentrations were measured by a glucose peroxidase method using a commercial kit (Glucose B-Test, Wako). Plasma insulin concentrations were determined by radioimmunoassay using a commercial kit (IRI Eiken, Eiken Chemical Co., Ltd., Tokyo).

Determination of Hepatic Plasma Flow Rate

In control and diabetic rats, hepatic plasma flow rates (Q_H) were determined using an ICG infusion method. Briefly, under ketamine–xylazine anesthesia (ketamine, 235 mg/kg i.m.; xylazine, 2.3 mg/kg, i.m.), the abdomen was opened through a midline incision and the hepatic vein of the left lobe was cannulated according to the method of Yokota *et al.* (13) for the collection of hepatic venous blood. The left femoral vein and left femoral artery also were cannulated with SP-31 tubing. The abdomen was sutured after the correct cannulation was confirmed, and the rats were kept in the Bolman cages. A saline solution of ICG was infused into the femoral vein at the rates of 1.3 and 0.86 μmol/hr in control and diabetic rats, respectively, using an automatic infusion pump (model KN, Natsume Seisakusho Co., Tokyo). At designated times (0.5, 1, 1.5, and 2 hr), blood samples (0.25 ml) were withdrawn from the hepatic vein and femoral artery. Plasma was separated from blood by centrifugation, and the ICG concentration in plasma was determined as described later. Hematocrits (Ht) of the blood samples were measured using the last arterial and hepatic venous blood samples and averaged. After the last blood samples were obtained, the whole liver was quickly excised, rinsed with saline, blotted dry on a filter paper, and weighed. The Q_H was determined as follows:

$$Q_H = IR/(Ca_{ss} - Cv_{ss}) \quad (1)$$

where IR is the infusion rate of ICG into the femoral vein, and Ca_{ss} and Cv_{ss} are the plasma ICG concentrations in the femoral arterial plasma and hepatic venous plasma at steady state, respectively.

Portal Vein Injection Technique

The unidirectional extraction of A_{14}-^{125}I-insulin in the liver was measured in control and diabetic rats, using the tissue sampling technique (14,15). Under ketamine–xylazine anesthesia, animals were placed in the supine position and laparotomized. After a portal vein was cannulated with a 27-gauge needle and the hepatic artery was ligated, an approximately 200-μl bolus of Ringer's–Hepes buffer [pH 7.4, 141 m*M* NaCl, 4 m*M* KCl, 2.8 m*M* $CaCl_2$, and 10 m*M* 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid], containing 0.5 μCi/ml A_{14}-^{125}I-insulin and 2.5 μCi/ml ^{3}H-water with and without 50 μ*M* unlabeled insulin, was rapidly injected within 0.5 sec into the portal vein. The ^{3}H-water was used as a highly diffusible internal reference of uptake. Eighteen seconds after portal injection, the portal vein was transected, and a portion of the right major lobe was immediately removed. The procedures of the sample treatment were the same as described previously (16). Briefly, aliquots of liver (~200 mg) and of the injection solution were counted in duplicate for simultaneous (^{125}I, ^{3}H) liquid scintillation counting according to the method described previously (17). The liver uptake index (LUI) was calculated as follows:

$$LUI\ (\%) = \frac{(^{125}I/^3H)\ dpm\ in\ liver}{(^{125}I/^3H)\ dpm\ in\ injection\ solution} \times 100 \quad (2)$$

The hepatic extraction of the test compound (A_{14}-^{125}I-insulin), E_T, was estimated from the following equation:

$$E_T = (LUI/100) \times E_R \quad (3)$$

where E_R is the percentage extraction of the reference compound (^{3}H-water) at 18 sec after rapid portal injection.

Determination of the Unidirectional Extraction of ^{3}H-Water

The operation procedures are the same as described above. A 200-μl solution of ^{3}H-water (0.5 μCi/ml) was injected and a portion of the right major lobe was immediately removed 18, 30, 45, 60, and 90 sec after portal injection. The unidirectional extraction of ^{3}H-water (E_R) was measured as follows:

$$E_R = (C_S/W_S)W_T(C_I \cdot V_I) \quad (4)$$

where C_S and C_I are the ^{3}H-water radioactivity in the liver sample and injection solution (dpm/ml), respectively; W_S and W_T represent the liver weights (g) of the liver sample used for counting and the whole liver, respectively; and V_I is the total volume injected into the portal vein. The E_R at time t (min) can be expressed as follows:

$$E_R = E_{R,max} \cdot e^{-K_B t} \quad (5)$$

where $E_{R,max}$ and K_B represent the maximal (initial) extrac-

tion of the reference and the rate constant (min^{-1}) of ^3H-water efflux from the liver during a circulation period after portal injection, respectively. The K_B and E_{max} values were estimated by fitting the observed E_R data to Eq. (5), using a nonlinear least-squares regression analysis (18). These two parameters may be used to determine hepatic blood flow rate (F) using the following relationship:

$$F = \frac{K_B \cdot V'}{E_{R,max}} \quad (6)$$

where V' is the liver–blood partition coefficient for ^3H-water, which is 0.91 ml/g for the rat liver (15).

Intravenous Bolus Injection of A_{14}-^{125}I-Insulin

Age-matched control and STZ-diabetic rats were lightly anesthetized with ether, and the femoral vein and left femoral artery were cannulated with polyethylene tubing (SP-31; 0.80-mm o.d. 0.40-mm i.d.; Natsume Seisakusho) for insulin administration and blood sampling, respectively. Before i.v. injection of A_{14}-^{125}I-insulin, control rats were kept in the Bolman cages and fasted for 48 hr for the monitoring of plasma concentrations of insulin and glucose. Diabetic rats were put into Bolman cages after the operation and stabilized for 1 hr. The control and diabetic conscious animals received an i.v. dose (3.2 μCi/kg) of A_{14}-^{125}I-insulin with and without a large excess of unlabeled insulin (8 U/kg), through the femoral vein. When unlabeled insulin was coadministered, glucose was constantly infused at a rate of 24 mg/min/kg to avoid hypoglycemia. Blood samples (approximately 0.3 ml) were withdrawn from the femoral artery at the designated times (2, 5, 10, 30, 60, and 120 min) after insulin administration and collected in polyethylene tubes. Serum was separated from blood by centrifugation, and the serum concentration of A_{14}-^{125}I-insulin was determined by TCA precipitation and HPLC, as described later.

Analytical Methods

ICG. The ICG concentrations in plasma were measured spectrophotometrically at 800 nm after 11 times dilution with water. A calibration curve was generated using plasma (from untreated rats) containing known quantities of ICG.

A_{14}-^{125}I-Insulin. Unchanged A_{14}-^{125}I-insulin in serum was measured by TCA precipitation and HPLC analysis methods (7). Since it is very laborious to analyze many biological samples by HPLC, we routinely employed the TCA-precipitation method, and the obtained TCA precipitability was converted into the percentage of unchanged A_{14}-^{125}I-insulin on HPLC, using a regression curve between these two methods.

Data Analysis

The Unidirectional Clearance of Insulin in the Liver. Since a portion of the unidirectional extraction represents simply distribution of label in the hepatic interstitial space, the hepatic extraction of A_{14}-^{125}I-insulin due to extravascular uptake, E_T', was calculated as follows:

$$E_T' = \frac{E_T - E_{suc}}{1 - E_{suc}} \quad (7)$$

where E_{suc} represents the apparent extracellular space in the liver, taken as 13% (14). Thus, the unidirectional extraction of A_{14}-^{125}I-insulin due to "specific" binding to surface receptors ($E_{T,sp}'$) can be expressed as follows:

$$E_{T,sp}' = E_T' - E_{T,ns}' \quad (8)$$

where $E_{T,ns}'$ represents the unidirectional extraction of A_{14}-^{125}I-insulin due to "nonspecific" binding, which was calculated from LUI_{ns} in rats injected with a tracer plus unlabeled insulin. Then, the unidirectional clearance (CL_{on}) of A_{14}-^{125}I-insulin, which represents the association of insulin to surface receptors, was calculated based on well-stirred [Eq. (9)] and sinusoidal perfusion [Eq. (10)] models, as follows:

$$CL_{on} = \frac{Q_H \cdot E_{T,sp}'}{1 - E_{T,sp}'} \quad (9)$$

$$CL_{on} = -Q_H \cdot \ln(1 - E_{T,sp}') \quad (10)$$

Pharmacokinetic Parameters of A_{14}-^{125}I-Insulin. Serum concentrations (C) of A_{14}-^{125}I-insulin were expressed as percentage of dose per milliliter serum as follows:

$$C = (\text{total cpm/ml serum}) \times (\text{intact percentage in serum})/\text{dose}/100 \quad (11)$$

where dose represents (intact cpm administered)/(kg body weight).

In control and diabetic rats, serum concentration versus time curves of A_{14}-^{125}I-insulin were analyzed by a noncompartmental moment method (19) with an adequate extrapolation of observed data to infinite time, and the total-body serum clearance (CL_{tot}) and steady-state apparent volume of distribution (Vd_{ss}) of A_{14}-^{125}I-insulin were calculated.

Hepatic Intrinsic Clearance of Insulin Due to a Specific Mechanism. Since the specific, receptor-mediated clearance of insulin can be attributed for the most part to the liver (6,8), the specific portion of CL_{tot} (named $CL_{tot,sp}$) was assumed to be included in the hepatic clearance. Subsequently, the apparent hepatic intrinsic clearance of A_{14}-^{125}I-insulin ($CL_{int,sp,app}$) due to a specific mechanism was calculated, based on well-stirred [Eq. (12)] and sinusoidal perfusion [Eq. (13)] models, as follows:

$$CL_{int,sp} = \frac{Q_H \cdot CL_{tot,sp}}{Q_H - CL_{tot,sp}} \quad (12)$$

$$CL_{int,sp} = -Q_H \cdot \ln(1 - CL_{tot,sp}/Q_H) \quad (13)$$

where $CL_{tot,sp}$ represents the difference in CL_{tot} between the dose of a tracer alone and the dose of a tracer plus 8 U/kg of unlabeled insulin.

RESULTS

Characteristics of Animals

The body weight, plasma glucose, and insulin levels of the control and STZ-diabetic rats at the time of the experiments are shown in Table I. Plasma glucose levels were significantly higher in STZ-diabetic rats than in control rats.

Table I. Comparison of Body Weight, Plasma Glucose, and Plasma Insulin Between Control and STZ-Diabetic Rats[a]

Characteristics	Control	Diabetic
Body weight (g)	297 ± 5 (11)	176 ± 9* (5)
Plasma glucose (mg/dl)	149 ± 10 (7)	717 ± 22* (5)
Plasma insulin (pmol/L)		
Femoral vein	135 ± 15 (10)	147 ± 13 (4)
Portal vein	378 ± 18 (6)	179 ± 9* (4)

[a] The data are expressed as the means ± SE. The numbers in parentheses represent the number of rats used.
* Significantly different from control rats ($P < 0.001$), as assessed by Student's t test.

Systemic insulin concentrations were low and very close between control and STZ-diabetic rats, while portal insulin concentrations were significantly higher in control rats.

Hepatic Plasma Flow Rate

As listed in Table II, hepatic plasma flow rate (Q_H) in STZ-diabetic rats (1.64 ml/min/g liver) was significantly higher than that in control rats (0.982 ml/min/g liver), while liver weight and hematocrit (Ht) were almost the same with each other.

Liver Uptake Index of A_{14}-^{125}I-Insulin

The percentage dose of ^{3}H-water extracted per gram of liver decreased monoexponentially (result not shown), and the obtained LUI parameters are listed in Table III. The CL_{on} was markedly elevated in STZ-diabetic rats compared with control rats, and the increase in CL_{on} was statistically significant, assuming that the E_R and F are constant in each group.

Pharmacokinetics of A_{14}-^{125}I-Insulin

Since the relationship of A_{14}-^{125}I-insulin intactness (%) in serum samples between the TCA-precipitation and the HPLC methods was essentially overlapping with that previously reported (7), the obtained TCA precipitability, which was routinely employed for the determination of A_{14}-^{125}I-insulin in serum, was converted to the percentage of intact insulin on HPLC using their correlation. Serum disappearance curves of A_{14}-^{125}I-insulin in control and STZ-diabetic rats after i.v. injection are presented in Figs. 1A and B. The CL_{tot} and Vd_{ss} at a tracer dose were significantly different between the control and the STZ-diabetic rats, while those at a high dose were not significantly different. The CL_{tot} of A_{14}-^{125}I-insulin at a tracer dose (23.3 ml/min/kg) in control rats was in good agreement with that (23.6 ml/min/kg) reported previously in normal rats using ^{3}H-insulin as a tracer (20). In both groups of rats, the CL_{tot} and Vd_{ss} of labeled insulin decreased significantly with a simultaneous injection of unlabeled insulin (8 U/kg). Expressed as units of milliliters per minute per gram of liver, the $CL_{tot,sp}$ and $CL_{int,sp}$ were elevated in diabetic rats by 46 and 40%, respectively. This is consistent with the study by Rabkin *et al.* (8), who observed 30 and 65% increases in the hepatic clearance (CL_H) and hepatic intrinsic clearance (CL_{int}) of immunoreactive insulin, respectively, in the perfused livers of diabetic rats. In this study, however, a statistical comparison of $CL_{tot,sp}$ or $CL_{int,sp}$ was not performed, because each parameter (i.e., Q_H and CL_{tot} at low and high doses) was determined in a different group of rats.

Table II. Comparison of Hepatic Plasma Flow Rate, Liver Weight, and Hematocrit Between Control and STZ-Diabetic Rats[a]

Characteristics	Control	Diabetic
Hepatic plasma flow rate (ml/min/g liver)	0.982 ± 0.124	1.64 ± 0.05*
Liver weight (g)	10.5 ± 0.3	9.64 ± 0.60
Hematocrit (Ht)	0.480 ± 0.012	0.459 ± 0.010

[a] The data are expressed as the means ± SE from three rats.
* Significantly different from control rats ($P < 0.001$), as assessed by Student's t test.

DISCUSSION

This study was designed to evaluate quantitatively the hepatic intrinsic clearance of insulin in diabetic states under an *in vivo* condition, where we have found a significant change in the hepatic plasma flow rate. Although the effect of hepatic blood flow rate on the elimination and distribution of drugs have been extensively studied (9,10,21), its effect on the hormone disposition has not been comprehensively understood due to the lack of quantitative analysis.

The portal injection technique revealed that CL_{on} is significantly higher in diabetic rats than in control rats (Table III). Since an intravenous injection of xylazine (0.5 mg) causes hypoinsulinemia for up to approximately 2 hr (22), the CL_{on} could be directly compared between control and diabetic rats without correction for the extracellular insulin concentration (C_e), as described in the Appendix. It is generally considered that target cells from STZ-diabetic rats exhibit increased binding of ^{125}I-insulin, due to an increase in surface receptor number (R_T) with no change in receptor affinity (1–4). Thus, it is likely that the increased CL_{on} is attributed, for the most part, to an increase in the R_T. The present study, therefore, has provided *in vivo* evidence for the "up-regulation" of surface insulin receptors in the livers of STZ-diabetic rats.

With regard to the pharmacokinetic parameters of A_{14}-^{125}I-insulin (Table IV), the large Vd_{ss} at a tracer dose and its significant decrease with coadministration of unlabeled insulin suggest that insulin is not only distributed to the extracellular fluid but also reversibly bound to its specific binding sites (receptors) in target tissues. Moreover, the CL_{tot} was reduced significantly in control and diabetic rats, by 51 and 64%, respectively, with a simultaneous injection of unlabeled insulin. Taken altogether, it is confirmed that saturable and receptor-mediated processes of tissue distribution and elimination are involved in the pharmacokinetics of A_{14}-^{125}I-insulin in both groups of rats.

Philippe *et al.* (5) demonstrated that the metabolic clearance rate of ^{125}I-insulin was elevated by 44% in STZ-diabetic rats and related this change to the increased binding of insulin to a specific receptor compartment in diabetic rats. However, since the previous study has not examined a change in hepatic blood flow rate or portal insulin concen-

Table III. Comparison of the LUI Parameters in Portal Injection Technique Between Control and STZ-Diabetic Rats

Compound	Parameter	Control	Diabetic
^{3}H-Water	$E_{R,max}$[a] (%)	73.2 ± 3.0	80.6 ± 11.5
	k_H[a] (min^{-1})	0.870 ± 0.069	1.21 ± 0.24
	F[b] (ml/min/g liver)	1.07	1.29
A_{14}-^{125}I-insulin[c]	LUI[d] (%)	145 ± 8	191 ± 10*
	LUI_{ns}[d] (%)	50.5 ± 4.0	54.9 ± 7.9
	E_T'[e] (%)	78.7 ± 5.2	96.3 ± 5.9
	$E_{T,ns}'$[e] (%)	17.6 ± 2.6	16.0 ± 4.5
	CL_{on}[f] (ml/min/g liver)		
	Well-stirred model	1.82 ± 0.38	5.56 ± 1.42*
	Sinusoidal perfusion model	1.03 ± 0.15	2.02 ± 0.34*

[a] Determined by Eq. (5) from a monoexponential decay curve of the ^{3}H-water extraction using a nonlinear least-squares regression analysis (18) and expressed as the mean ± SD.
[b] Calculated by Eq. (6), assuming that V' is 0.91 ml/g (15).
[c] The data are expressed as the mean ± SE. The numbers of rats used are four and six for control or diabetic rats, respectively.
[d] Calculated by Eq. (2).
[e] Calculated by Eq. (7).
[f] Calculated by Eqs. (9) and (10) for well-stirred and sinusoidal perfusion models, respectively.
* Significantly different from control rats ($P < 0.05$), as assessed by Student's t test.

trations, it has remained uncertain whether or not the hepatic intrinsic clearance (CL_{int}) of insulin was actually altered in STZ-diabetic rats. In this study, a significant increase in Q_H was observed in STZ-diabetic rats, despite the fact that the liver weight was not changed (Table II). This observation is consistent with previous reports demonstrating diabetes-induced increases in permeation of vessels and tissues by various tracer in diabetic humans and animals (23,24). On the other hand, the LUI method indicated that, under ketamine anesthesia, the hepatic blood flow rate (F) was 21% higher in diabetic rats than in control rats (Table III) and that these values were smaller than the hepatic blood flow rates measured with the ICG infusion, i.e., 1.89 and 3.03 ml/min/g liver in control and diabetic rats, calculated from $Q_H/(1 - Ht)$. This discrepancy could be explained by the effect of ketamine anesthesia on the hepatic blood flow rate, because Pardridge and Fiere (25) previously reported a 44% reduction of cerebral blood flow rate in the ketamine-anesthetized rats, as compared with conscious rats.

In addition to the hepatic plasma flow, we have to consider the difference in portal insulin concentration (C_p) between control and diabetic rats. In order to minimize such a difference, we attempted to minimize the C_p in control rats, so that an efficient hepatic extraction of insulin could be observed. McCarroll and Buchanan (26) reported mean extraction ratios of 22.2% for perfused livers of fed rats and 42.2% for preparations taken from fasted for 72 hr, supporting the view that the ability of the liver to extract insulin from the portal blood increases during fasting. Therefore, we used 48-hr-fasted rats as control animal, in which the C_p was minimized but still higher than that in diabetic rats (Table I). When $CL_{int,sp}$ was corrected for the difference in C_p using Eqs. (A1)–(A5) in the Appendix, the $CL_{int,sp,corr}$ was shown to be higher in diabetic rats than in control rats by 24 and 31%, based on well-stirred and sinusoidal perfusion models, respectively. In any event, it is important to realize that an overall increase in CL_{tot} of A_{14}-^{125}I-insulin in STZ-diabetic rats could be interpreted as a result of the changes in not only $CL_{int,sp}$ and C_e but also Q_H (as a major factor).

In general, the $CL_{int,sp}$ can be expressed as follows:

$$CL_{int,sp} = k_{on} \cdot R_T \cdot k_e/(k_e + k_{off}) \quad (14)$$

where k_e represents the endocytotic rate constant of insulin, and k_{on} and k_{off} represent the association and dissociation rate constants of insulin-receptor binding, respectively. Equation (14) indicates that an increase in R_T (or CL_{on}) and a decrease in k_e would affect $CL_{int,sp}$ (and also CL_{int}) in the opposite direction. Thus, the slight increase in $CL_{int,sp}$, which is inconsistent with the large increase in CL_{on}, might be accounted for by an decrease in k_e. This is a likely explanation, since there was a delay in the preclustering of asialoorosomucoid receptors in the hepatocytes from diabetic rats (27). Therefore, further studies are required to clarify whether or not the k_e of insulin receptors is reduced in STZ-diabetic rats, using such as isolated hepatocytes.

In conclusion, from the significant increases in CL_{tot} and Q_H, we have provided *in vivo* evidence for a slight increase in $CL_{int,sp}$ of insulin in STZ-diabetic rats, which may be explained by counteracting effects of an increased surface

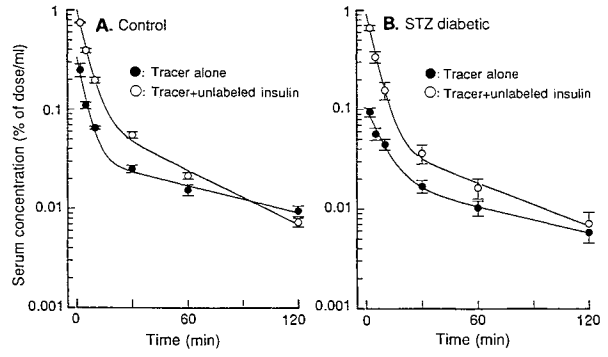

Fig. 1. Serum disappearance curves of A_{14}-^{125}I-insulin in control (A) and STZ-diabetic (B) rats after intravenous bolus injection (3.2 μCi/kg) with (○) and without (●) 8 U/kg of unlabeled insulin. Each point and vertical bar represent the mean ± SE (n = 3–6). Solid lines were drawn by a nonlinear least-squares regression analysis (18) using a biexponential equation.

Table IV. Comparison of Pharmacokinetic Parameters Between Control and STZ-Diabetic Rats[a]

Parameter	Dose	Control	Diabetic
CL_{tot} (ml/min/kg)	Tracer dose	23.3 ± 1.8 (6)[b]	41.9 ± 8.5* (4)
	+ unlabeled insulin	11.4 ± 0.5 (3)	14.9 ± 2.4 (3)
Vd_{ss} (ml/kg)	Tracer dose	1546 ± 68 (6)	2634 ± 352* (4)
	+ unlabeled insulin	278 ± 18 (3)	399 ± 81 (3)
$CL_{tot,sp}$[c]			
ml/min/kg		11.9	27.0 (227%)[d]
ml/min/g liver		0.337	0.493 (146%)
$CL_{int,sp}$[e] (ml/min/g liver)			
Well-stirred model		0.513	0.705 (137%)
Sinusoidal perfusion model		0.408	0.585 (143%)
$Vd_{ss,sp}$[c] (ml/kg)		1268	2235 (176%)

[a] Using the serum concentrations of A_{14}-^{125}I-insulin after i.v. injection, the CL_{tot} and Vd_{ss} were determined by a noncompartmental moment analysis (19) and are expressed as mean ± SE.
[b] The numbers in parentheses represent the number of rats used.
[c] Determined as the difference of CL_{tot} or Vd_{ss} between the dose of a tracer only and the dose of a tracer plus 8 U/kg of unlabeled insulin.
[d] The numbers in parentheses represent the ratio (%) of the parameters in STZ-diabetic rats to those in control rats.
[e] Determined by Eqs. (12) and (13) for well-stirred and sinusoidal perfusion models, respectively.
* Significantly different from control rats ($P < 0.01$), as assessed by Student's t test.

receptor number (i.e., up-regulation) and a decreased endocytotic rate constant for insulin–receptor complex in the liver. Since the pharmacokinetic implications of physiological parameters (e.g., CL_{int}, Q_H, and extracellular insulin concentration) appears to be lacking in the previous studies on insulin pharmacokinetics, the present study would be viewed as a physiological approach to the quantitative evaluation of receptor-mediated hepatic clearance of insulin.

APPENDIX

Equations to Calculate the Fraction of Unoccupied Surface Receptors in the Liver

Considering the situation where the binding of extracellular insulin with surface receptors is in equilibrium, the fraction of unoccupied surface receptors (R) in the liver is given as follows:

$$R = 1 - C_e/(K_d + C_e) \quad \text{(A1)}$$

where K_d represents the dissociation constant of insulin–receptor binding, and C_e the average extracellular insulin concentration in the liver. Since the major portion of blood presented to the liver was from portal vein, the C_e can be approximately calculated using the well-stirred and sinusoidal perfusion models, as follows:

$$C_e \cong C_p \cdot (1 - E_H) \quad \text{(A2)}$$
$$C_e \cong -C_p \cdot E_H/\ln(1 - E_H) \quad \text{(A3)}$$

where C_p is the portal insulin concentration, and the hepatic extraction ratio (E_H) of insulin at steady state is obtained by

$$E_H = Q_H/CL_H \quad \text{(A4)}$$

where Q_H and CL_H represent the hepatic plasma flow and hepatic insulin clearance, respectively. In this study, CL_H was taken as the specific portion of the total clearance, $CL_{tot,sp}$.

Since A_{14}-^{125}I-insulin is known to have the same receptor binding affinity with native insulin (29) and exhibits receptor binding to hepatocytes and isolated liver plasma membranes with a K_d of approximately 1 nM (30), we employed a representative K_d of 1 nM in Eq. (A1). Provided that an i.v. dose of A_{14}-^{125}I-insulin is so low that A_{14}-^{125}I-insulin could not interfere with the binding of endogenous insulin to surface receptors in the liver, the hepatic intrinsic clearance of insulin ($CL_{int,sp}$) can be corrected for the difference in C_e, as follows:

$$CL_{int,sp,corr} = CL_{int,sp}/R \quad \text{(A5)}$$

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