Increased Clearance and Degradation of [^{3}H]Insulin in Streptozotocin Diabetic Rats

ROLE OF THE INSULIN-RECEPTOR COMPARTMENT

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ABSTRACT The role of the insulin-receptor compartment in the pharmacokinetics of intravenously injected insulin in rats was studied. Since streptozotocin-diabetes in rats results in increased insulin binding to tissues in vitro, insulin pharmacokinetics in streptozotocin-diabetic rats were compared to controls, using semisynthetic [^{3}H]insulin as the tracer. The initial distribution volume for [^{3}H]insulin was elevated by 60% in diabetic rats. By contrast, no difference in initial distribution volume for [^{14}C]inulin was observed, and the absolute values were lower than those found for [^{3}H]insulin. The metabolic clearance rate of [^{3}H]insulin was elevated by 44% in diabetic rats. That these differences were the result of increased binding of insulin to a specific receptor compartment in diabetic rats was shown by three additional experiments. The first involved receptor saturation by injection of 10 U native insulin 2 min before the tracer injection, resulting in identical [^{3}H]insulin disappearance rates in the two groups of rats. The second consisted of displacing [^{3}H]insulin from receptors by injecting 10 U unlabeled insulin 6 min after the tracer injection. Displacement of intact [^{3}H]insulin from receptors and subsequent reappearance in the circulation occurred in both control and diabetic animals; however, such displacement was 25% greater in the diabetic rats. Finally, treatment of diabetic rats with insulin for 8 d normalized [^{3}H]insulin clearance even though the tracer was injected at a time when the animals were again hyperglycemic and hypoinsulinemic. This suggests that down-regulation of insulin receptors had occurred during insulin therapy. These results confirm that a specific compartment for insulin exists (the insulin-receptor compartment) and that this compartment plays an important role in insulin clearance.

INTRODUCTION

Target cells from diabetic animals have an increased binding capacity for insulin compared with control animals. This observation is taken as evidence for an increased number of receptor sites on, for example, liver plasma membranes (1, 2) or adipocytes (3) from streptozotocin-induced diabetic rats. Similarly, muscle cells from experimentally hypoinsulinemic diabetic mice (4) and rats (5) display increased insulin binding. The observation in all three tissues is compatible with the widely accepted hypothesis of regulation of insulin receptors by ambient insulin concentration in vitro (6) and in vivo (7).

Zeleznik and Roth (8) and others (9) have estimated in vivo that >50% of extrapancreatic insulin is bound to receptors under controlled physiological conditions. It has also been shown that receptors in vivo display selectivity for insulin of different species comparable to that observed in vitro (8). It may be concluded from these experiments that a receptor compartment for insulin exists in vivo. We suggest that this compartment may profoundly influence insulin metabolism and have examined this possibility experimentally.

Our study was thus designed to assess the effect of increased insulin binding capacity (associated with persistently low plasma-insulin concentrations, as in streptozotocin-induced diabetes) upon the disappearance of insulin from blood as compared to that in con-

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TABLE I
Body Weight, Plasma Glucose, and Insulin

	Body weight		Plasma glucose	Plasma insulin
	Before streptozotocin injection (basal)	At end of treatment period (before experimental test)		
	g	*g*	*mg/100 ml*	*μU/ml*
Control rats (n = 24)	295±8	330±4	130±3	28.2±2
Untreated diabetic rats (n = 20)	316±8*	290±4*	528±23*	6.5±1*
Insulin-treated diabetic rats (n = 4)				
During treatment	315±5*	350±5*	205±4*	96*
Before test			606±41*	14.8±1*

Data are presented as mean±SEM.
* Difference vs. controls statistically significant ($P < 0.05$).

trol animals. To this end, radioactively labeled insulin was injected intravenously and its disappearance from the circulation was measured. The tracer used, semisynthetic [^{3}H]insulin (10, 11), has been shown to be indistinguishable from native insulin by chemical, immunological, and biological criteria (10–12).

METHODS

Semisynthetic [^{3}H]insulin

Semisynthetic [^{3}H]insulin at a specific radioactivity of 10 Ci/mmol was prepared by the method of Halban and Offord (10). In brief, the preparation involves the replacement of the aminoterminal residue of the B chain of pork insulin, phenylalanine, by [^{3}H]phenylalanine, followed by two purification steps to remove excess [^{3}H]phenylalanine and to separate intact insulin from damaged material (10, 11). 94–96% of the radioactivity was associated with intact insulin as determined by gel chromatography, immunoprecipitation, and immunoassay. It was shown to be biologically effective and comparable to native insulin by in vivo bioassay in the rat.

Experimental animals

Adult male Wistar rats, fed standard purina chow and weighing 300–330 g were used for all experiments. Diabetes was induced by a single intravenous injection (dorsal tail vein) of streptozotocin (50 mg/kg) dissolved immediately before use in 0.01 M citrate buffer, pH 4.0 (13). Control animals were injected with buffer alone. The streptozotocin-injected rats developed glycosuria, polydipsia, and hyperglycemia within 48 h. Plasma glucose and insulin were measured on day 5 after streptozotocin injection (Table I). Only those rats with a basal plasma-glucose level of 300 mg/dl or higher and a plasma-insulin concentration not exceeding half the mean control values, were included in the study. The hepatic and renal functions of the diabetic animals were also studied (Table II). Despite a small increase in plasma-hepatic transaminases, there were no changes in levels of bilirubin, nitrogen, electrolytes, or kidney mass. The rats were assigned to three groups: (*a*) control, (*b*) untreated diabetic, and (*c*) treated diabetic rats. Each morning the treated group received 6 U Actrapid Monocomponent and 10–14 U Lente Monocomponent insulin subcutaneously for 8 d, beginning 8 d after the administration of streptozotocin. This dose of insulin was selected to restore plasma glucose, urine volume, and body weight to values as near to normal as possible (Table I). The last injection was given on the day (about 24 h) before the experiment. At the time of the experimental test the animals were again hypoinsulinemic and hyperglycemic (Table I).

Catheterization and blood sampling during measurement of [^{3}H]insulin disappearance

Unanesthetized rats. 2–3 wk before tracer injection, permanent cardiac catheters were implanted via the jugular vein according to the method of Steffens (14). The jugular catheter ran subcutaneously to the vault of the skull and was then exteriorized via a headpiece of dental cement. This headpiece was anchored by jeweler's screws to the skull. The catheters were filled with saline containing polyvinylpyrrolidone (0.7 g/ml) and heparin (500 U/ml). The polyvinylpyrrolidone-heparin-saline mixture was aspirated daily and the whole catheter was flushed out with heparinized saline and then refilled with polyvinylpyrrolidone-heparin-saline solution.

The animals were housed individually, and the [^{3}H]insulin disappearance tests were performed 2–3 wk after implan-

TABLE II
Metabolic Markers for Hepatic and Kidney Function

	Control rats*	Diabetic rats*
Glutamate-pyruvate transaminase, *U/liter*	30±2	40±3‡
Glutamate-oxaloacetate transaminase, *U/liter*	45±4	58±5‡
Bilirubin, *μmol/liter*	2±0.1	2±0.1
Na, *mmol/liter*	143±2	140±2
K, *mmol/liter*	4.6±0.3	4.3±0.2
Urea, *mmol/liter*	8.4±0.6	8.3±0.5
Kidney weight, *g/100g body wt*	0.47±0.2	0.5±0.3

Data are presented as means±SEM.
* n = 4.
‡ Difference vs. controls statistically significant ($P < 0.05$).

tation of the catheters, when the rats had regained the weight they had lost immediately after surgery. During that time, they were trained to adapt to the blood sampling procedure. The tests were done in the morning, after the rats had free access to food and water during the previous night. Before each test a small extension tube was attached to the cardiac catheter; the tests were begun 10 min after these manipulations. A pretest blood sample was taken for the determination of plasma glucose and insulin. At time 0, the insulin tracer, semisynthetic [^{3}H]insulin, at a specific radioactivity of 10 Ci/mmol, was rapidly injected (1 μCi or 14.5 mU/100 g of body wt, dissolved in 0.2 ml 0.9% NaCl [wt/vol] containing 2.5 mg/ml bovine serum albumin, pH 7.4) through the cardiac catheter. The catheter was flushed through several times with saline and heparin (50 U/ml) to remove any trace of radioactivity. Blood samples were taken through the same catheter at sampling times 1, 5, 8, 10, 15, 30, and 60 min; in some experiments only the rapid disappearance phase was investigated and the sampling times were limited to 1, 2, 3, 5, 8, 10, and 15 min. Each blood sample (400 μl) was transferred to a heparinized microfuge tube and plasma was separated by centrifugation. Total blood taken during the experimental period amounted to 2.4 ml. The volume of each blood sample was replaced by an equivalent volume of 0.9% NaCl (wt/vol)-bovine serum albumin 2.5 mg/ml.

Anesthetized rats. Some of the [^{3}H]insulin disappearance experiments were performed under sodium pentobarbital anesthesia (5 mg/100 g of body wt). Both jugular veins were then exposed and catheterized: semisynthetic [^{3}H]insulin was injected in one and the blood samples were obtained from the second. It has been shown, using a similar protocol, that anesthesia does not affect insulin clearance rates (15).

Determination of [^{3}H]insulin in plasma

After blood centrifugation, the plasma samples were stored frozen. Radioactivity was determined in a Beckman Liquid Scintillation Counter (LS 8,000, Beckman Instruments, Fullerton, Calif.) using Biofluor as the scintillant. To separate intact labeled insulin from degradation products, plasma samples were chromatographed as previously described (15) on G-50 Fine Sephadex (column dimension 0.8 × 70 cm) using 0.2 M glycine, 2.5 mg/ml bovine serum albumin, pH 8.8, as the elution buffer. The radioactivity eluted as three distinct peaks. The middle peak eluted in the same volume as native insulin and has previously been shown to consist of material that is immunoprecipitable by anti-insulin serum (12). This contrasts with the first and third peaks, which are not immunoprecipitable and which consist of high and low molecular weight degradation products, respectively. The high molecular weight degradation products are generated by reincorporation of [^{3}H]phenylalanine (liberated by proteolytic degradation of [^{3}H]insulin) into newly synthesized proteins (12, 15, 16). The low molecular weight products are essentially [^{3}H]phenylalanine. Peak size was calculated planimetrically and was expressed as percentage of total radioactivity eluted from the column. The recovery of radioactivity from the column was >90%. [^{3}H]insulin radioactivity in plasma was determined by multiplying the total sample radioactivity by the percentage radioactivity eluting with the insulin peak. The percentage of total radioactivity attributable to intact [^{3}H]insulin was also estimated by immunoprecipitation. 100 μl plasma + 0.5 ml phosphate buffer + 20 μl antiporcine insulin serum in excess were incubated for 3 d at 4°C. The antigen-antibody complexes were then precipitated by the addition of 0.5 ml polyethylene glycol 20% (17) and the samples were centrifuged at 1,800 *g* for 45 min; radioactivity was counted in the pellet and in the supernatant fluid).

Initial distribution volume as determined by [^{3}H]insulin and [^{14}C]inulin

Initial distribution volume (IDV)[1] of both normal and diabetic IDV rats was determined after a single intravenous injection of [^{14}C]inulin mixed with [^{3}H]insulin. The IDV represents the space in which the tracer is immediately distributed and is calculated from the ratio of the injected dose and the tracer concentration at time 0 (18, 19).

The [^{14}C]inulin (1.5 μCi/100g) and the [^{3}H]insulin (1 μCi/100g) were injected intravenously in a total volume of 0.3 ml 0.9% NaCl using the same protocol as described above for [^{3}H]insulin. Blood samples (0.1 ml) were taken at times 1, 2, 3, 5, 6, 7, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min after injection. Total ^{14}C-radioactivity and the concentration of intact [^{3}H]insulin were then determined, the two isotopes being detected simultaneously in the scintillation counter.

Data analysis

The kinetic parameters were derived from the plasma disappearance curves of labeled insulin (Fig. 1), using a 2-compartmental model (20). This model was adopted to permit full use of the information provided by the kinetic studies. The model consists of a compartment (compartment 1), accessible to sampling, in which the tracer was injected and which corresponds to the IDV. From this compartment [^{3}H]insulin distributes to compartment 2 with a rate constant $K_{2,1}$. This compartment participates in the slow phase of insulin distribution and in its degradation; the rate of return of insulin to compartment 1 is described by the constant $K_{1,2}$. Insulin will eventually leave the system (through degradation): this is the fractional irreversible loss of insulin from plasma, and is defined kinetically by the constant $K_{0,2}$. Since urinary clearance of [^{3}H]insulin equals 0 (as measured by trichloroacetic acid [TCA]-insoluble radioactivity of the urine after the 60-min tests), it is not considered in this model. The metabolic clearance rate was estimated by multiplying the irreversible fractional loss rate by the plasma volume (calculated from the IDV).

Assays and statistical methods

Plasma glucose concentration was measured by the glucose oxidase method. Insulin was measured by radioimmunoassay (21) using rat insulin as standard and guinea pig antiporcine insulin serum. Student's unpaired *t* test was used to evaluate differences between groups.

Materials

Streptozotocin was a gift from Dr. W. Dulin (Upjohn Co., Kalamazoo, Mich.). Rat insulin was obtained from Dr. Schlichtkrull, (Novo Research Institute, Bagsvaerd, Denmark). Guinea pig antiserum against porcine insulin was a gift from Dr. H. H. Schöne (Farbwerke Hoechst, Frankfurt, West Germany). Bovine serum albumin was from Behringweke A.G., Marburg/Lahn, West Germany; Biofluor from New England Nuclear Corp., Boston, Mass.; Sephadex from Pharmacia Fine Chemicals, Uppsala, Sweden. Inulin-[^{14}C]-carboxylic acid (specific radioactivity 5–15 mCi/mmol) from Radiochemical Centre Ltd., Amersham, Buckinghamshire, England; and insulin used for treatment was from Novo Industri, Copenhagen, Denmark.

[1] *Abbreviation used in this paper*: IDV, initial distribution volume.

TABLE III

Kinetic Parameters for Intravenously Injected [^{3}H]Insulin in Control and Diabetic Rats

Parameters	Control rats*	Diabetic rats*	*P* value‡
IDV for [^{14}C]inulin, *ml/kg body wt*	44±6	52±7	NS
IDV for [^{3}H]insulin, *ml/kg body wt*	62±8	99±12	<0.005
IDV for [^{3}H]insulin after presaturation with 10 U unlabeled insulin, *ml/kg body wt*	46±7	55±5	NS
$K_{2,1}$	2.53±0.07	3.19±0.09	<0.005
$K_{1,2}$	0.14±0.01	0.14±0.02	NS
$K_{0,2}$§	0.29±0.01	0.36±0.02	<0.05
Metabolic clearance rate, *ml/kg/min*	12.9±0.5	18.6±1.1	<0.005

Data are expressed per kilogram of body weight and presented as mean±SEM.

* n = 4.

‡ P values are for level of significance for control vs. diabetic rats.

§ Fractional irreversible loss of [^{3}H]insulin from plasma.

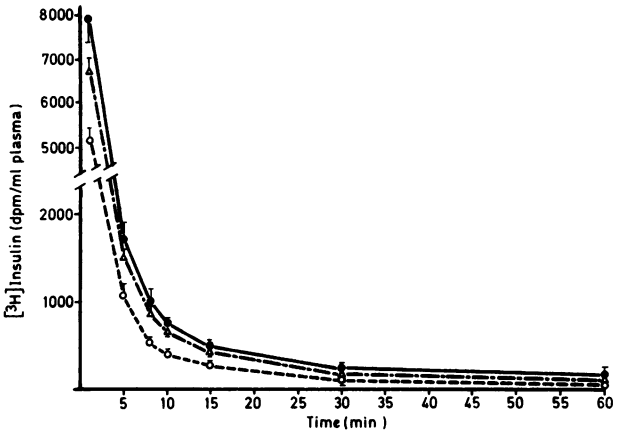

FIGURE 1 Disappearance of [^{3}H]insulin from plasma after intravenous injection (1 μCi; 14.5 mU/100 g of body wt) in four control rats (●), four untreated diabetic rats (○), and four insulin-treated diabetic rats (△). [^{3}H]insulin was separated from its labeled degradation products by gel chromatography and expressed as dpm/ml. All results are normalized as in an injection of 10^5 dpm [^{3}H]insulin/100 g body wt. The results are expressed as means±SEM. Differences between controls and untreated diabetic rats are statistically significant ($P < 0.05$) for all time points. There are no statistically significant differences between controls and treated diabetic rats.

RESULTS

Effect of streptozotocin diabetes on the IDV of [^{14}C]*inulin.* IDV was determined in both control and diabetic rats, after the intravenous injection of [^{14}C]inulin, as described in Methods.

The mean IDV of control rats was not significantly different from that of diabetic animals (Table III). The values agreed with previously reported data, estimating the IDV at around 4–5% of the body weight (22).

Effect of streptozotocin-induced diabetes on the metabolic clearance rate of [^{3}H]*insulin.* As shown in Fig. 1, insulin disappearance from the circulation is more rapid in diabetic rats than in controls. This difference can clearly be seen for all time points.

The data of the plasma-insulin disappearance curves were analyzed by computer, using a 2-compartmental model (20). Experimental points were in close association with the computer model-derived points. The kinetic parameters of both control and diabetic rats are seen in Table III. $K_{2,1}$ represents the rate constant for passage from compartment 1 (the compartment into which the insulin is injected) to compartment 2. This rate constant is greater in the diabetic rats. By contrast, $K_{1,2}$, which is the rate constant for return of insulin from compartment 2 to compartment 1, is similar in both groups. The functional irreversible loss of [^{3}H]insulin from the plasma, defined by the rate constant $K_{0,2}$ is again faster in the diabetic rats, suggesting that [^{3}H]-insulin is degraded more rapidly in these animals.

When the metabolic clearance rate was estimated from the computer-derived parameters, it was observed that [^{3}H]insulin clearance of diabetic rats was 144% that of the control rats.

Of interest was the estimation of the IDV after the injection of [^{3}H]insulin. As seen in Table III the IDV of the diabetic rats was 60% larger if compared to controls. The values for the IDV for [^{3}H]insulin were higher than the corresponding values for [^{14}C]inulin. The IDV may be defined as the space in which a given molecule is immediately distributed. Assuming that insulin is rapidly bound to receptors in vivo (see above), any difference between the IDV for insulin and that observed for inulin will be related to the specific binding of insulin to the receptor compartment. If the IDV for [^{14}C]-inulin is subtracted from that found for [^{3}H]insulin in order to estimate the receptor-compartment component of insulin IDV, then it can be seen that the receptor compartment for diabetics is 2.5–3 times that measured in controls.

The reappearance of radioactivity in the form of low molecular weight degradation products 5 min after injection can be seen in Fig. 2. There is a significant difference between the diabetic and control rats at 8, 10, and 15 min. The concentration of high molecular weight products, which have been shown to be due to the reincorporation of [^{3}H]phenylalanine into newly synthesized protein (15, 16), is similar in both groups.

Effects of insulin treatment. Because accelerated clearance of [^{3}H]insulin from diabetic rats may be the result of increased binding to receptors caused by the

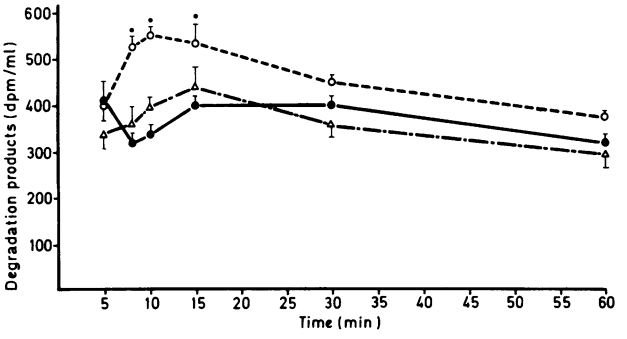

FIGURE 2 Low molecular weight degradation products of insulin in plasma after intravenous injection of [^{3}H]insulin in four control rats (●), four untreated diabetic rats (○), and four insulin-treated diabetic rats (△). Degradation products were separated from labeled insulin by gel chromatography and are expressed as dpm/ml. All results are normalized as for an injection of 10^5 dpm [^{3}H]insulin/100 g body wt. The results are expressed as means±SEM. * Significant difference ($P < 0.05$) between untreated diabetic rats and controls.

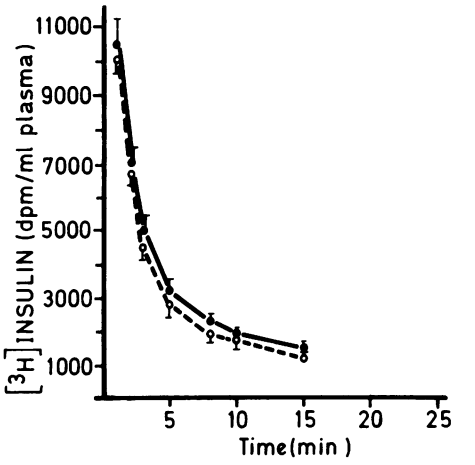

FIGURE 3 [^{3}H]insulin disappearance from plasma after intravenous injection in rats; effects of presaturation of insulin receptors by native insulin: 10 U of native insulin was injected 2 min before [^{3}H]insulin in four control rats (●), and in four untreated diabetic rats (○). [^{3}H]insulin is expressed as in Fig. 1. Means±SEM are shown.

chronic hypoinsulinemic state of these animals, an attempt was made to "down-regulate" (6, 7) insulin binding capacity by insulin therapy.

Glycemia, urine volume, and progression of body weight were followed during insulin treatment (Table 1). The dosage of insulin was adjusted to restore urinary volume to control levels. This required 6 U Actrapid + 12–14 U Lente/d, indicating that the streptozotocin-induced diabetic rats were resistant to insulin action. In comparison with untreated diabetic rats, blood glucose was lowered but was still significantly higher than that of controls (Table I). [^{3}H]insulin was injected 24 h after the last subcutaneous injection of exogenous insulin. At the time of the tracer injection, the animals were again hypoinsulinemic and hyperglycemic (Table I, bottom line). The insulin-receptor compartment was thus not presaturated with unlabeled insulin at the time of the experiment.

The effects of treatment on insulin disappearance are shown in Fig. 1. It can be seen that after insulin treatment the rate of disappearance of [^{3}H]insulin is comparable to that of controls. The effects are on both plasma total radioactivity and on percentage of intact [^{3}H]insulin, which was totally restored to normal. Consequently, the absolute amount of degradation products at 8, 10, and 15 min also fell to control levels (Fig. 2).

Presaturation of receptors. In a further attempt to evaluate the influence of the receptor compartment on the increased rate of [^{3}H]insulin clearance from plasma of diabetic rats, 10 U of unlabeled pork insulin was injected intravenously before the injection of the tracer to presaturate the receptors (8).

The insulin disappearance rates for both normal and diabetic animals were markedly reduced by the injection of 10 U of native insulin (compare Figs. 1 and 3). However, they were not reduced to the same extent for both groups, the most dramatic change being that observed in diabetic rats. The reduction in the latter group superimposed the insulin disappearance curve upon that of the controls. Again, no difference could be observed in the concentration of degradation products between control and diabetic rats following presaturation of the receptors.

These results are compatible with the existence of a specific compartment for receptor-bound insulin. The increased binding capacity of this compartment in diabetic rats would thus be overcome by presaturation. Indeed, presaturation with 10 U of unlabeled insulin could be expected to block all binding of tracer during a short period of time (after which, clearance of the unlabeled insulin will reexpose insulin receptors). In accordance with this suggestion is not only the reduced rate of clearance observed for both groups of rats, but also the reduction found in the IDV for [^{3}H]insulin. The IDV for insulin for the control animals in these series of experiments fell from 62±8 to 46±7 ml/kg, whereas in the diabetic animals, the value of 99±12 was lowered to 55±5 ml/kg after presaturation with unlabeled insulin (Table III). Thus, after presaturation of the insulin-receptor compartment, there was no longer a significant difference between the IDV for [^{14}C]inulin and [^{3}H]insulin in either group of rats (Table III), indicating that the insulin-specific compartment of [^{3}H]insulin distribution was indeed fully presaturated.

Reversibility of insulin binding to the receptor compartment. To displace any intact [^{3}H]insulin reversibly bound to receptors, 10 U of unlabeled insulin was injected intravenously 6 min after the injection of

the tracer. There was an immediate increase of insulin radioactivity due to increased total plasma radioactivity and to an increase in the percentage of intact [^{3}H]insulin. This follows from the observation that in both control and diabetic rats the [^{3}H]insulin concentration in the plasma 2 min after injection of the unlabeled insulin is greater than that observed at the time of the unlabeled insulin injection (i.e. 6 min after the tracer injection). This net increase in [^{3}H]insulin concentration indicates that labeled material has been displaced, presumably from the receptor compartment, and has subsequently reappeared in the circulation. Furthermore, as shown in Fig. 4, the amount of [^{3}H]insulin that was displaced and then reappeared in the circulation was much greater in diabetic animals. The most striking effect was seen just after the unlabeled insulin injection (between 6 and 10 min). Labeled insulin displaced during that period, as measured by the surface area above the control insulin disappearance curve of both groups, was 25% greater in diabetic rats than in normal animals, suggesting again that the receptor compartment in diabetic rats is larger. Clearly the injection of 10 U unlabeled insulin will also reduce the rate of disappearance of [^{3}H]insulin (Fig. 3), and this is reflected in the observation that after 10 min the disappearance rates of the labeled insulin were similar in both groups.

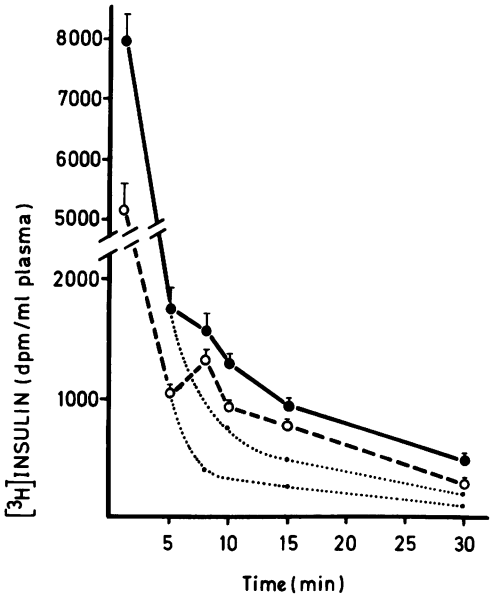

FIGURE 4 [^{3}H]insulin disappearance from plasma after intravenous injection in rats; displacement of intact [^{3}H]-insulin by native insulin: 10 U of native insulin was injected 6 min after tracer injection in four normal rats (●) and four untreated diabetic rats (○). ····· refers to control insulin disappearance curve for each group of rats when no native insulin was injected. [^{3}H]insulin is expressed as in Fig. 1. Means±SEM are shown.

DISCUSSION

Studies on the pharmacokinetics of insulin have been greatly facilitated by the availability of radioactively labeled insulin tracers. However, interpretation of the results of these studies has been severely handicapped by doubts concerning the authenticity of the radioiodinated insulins used in the majority of these studies (23, 24, 27–29). Although new techniques for labeling and purifying radioiodinated insulins (30–32) have yielded tracers of value for pharmacokinetic studies, even these preparations may not be fully homogenous. To overcome these problems we have used, as in previous studies (15, 33–35), semisynthetic [^{3}H]insulin (10, 11). This tracer is labeled uniquely on the amino terminal phenylalanine of the insulin B chain. It is identical to native insulin, with the exception of the isotopic replacement of tritium for hydrogen on this phenylalanine residue.

The results of this study demonstrate that streptozotocin-induced diabetes in rats leads to an increased metabolic clearance rate of [^{3}H]insulin compared with control animals. It should be noted that the methodology used in the present study imposes certain limitations on the quantitative interpretation of the results. Thus, the experimental design necessitated taking relatively small volumes of blood (400 μl) for each time point. To ensure that there was enough radioactivity in each sample for later analysis, the amount of [^{3}H]insulin injected corresponded to significant amounts of insulin (14.5 mU/100 g of body wt). Indeed, the [^{3}H]-insulin injected resulted in a blood glucose depression of 30±5% of the pretest concentration, after 10 and 15 min. Despite decreased blood glucose and increased plasma insulin, the disappearance of [^{3}H]insulin from the plasma should not have been affected to any significant extent, provided that the insulin-removal mechanisms remained unsaturated over the range of insulin variations induced. We think that this is most likely the case because of reports that disappearance of ^{125}I-insulin under basal conditions and during glucose infusion was the same (25) and the immunoreactive insulin curves obtained after infusing large amounts of either unlabeled or labeled hormone (15, 23–25) were essentially the same, apart from a scale factor. Finally, the results in the present studies show that changes in IDV for [^{3}H]insulin occur following presaturation of the receptor compartment with native insulin (Table III) and that labeled material can be displaced from the receptor compartment of unlabeled insulin (Fig. 4). These effects would not be observed if the receptor compartment were already saturated by the injection of the labeled product alone.

Streptozotocin-induced diabetes in rats was chosen as the model for this study, since the metabolic characteristics of the disease are well defined (36) and the

resulting hypoinsulinemia has been shown to lead to increased insulin receptor binding in vitro (1–5). From our data, we can conclude that this increased binding is the principal factor in the accelerated insulin clearance in diabetic animals. Thus, we demonstrate, in accordance with Zeleznik and Roth (8), that a body compartment exists with characteristics of specific cell surface receptors. Indeed, like receptors studied in vitro, this compartment shows saturability and reversibility. In addition, we have shown that the size of the compartment is important when considering plasma-insulin clearance.

The results are consistent with a rapid and partially reversible sequestration of insulin by the receptor compartment. The importance of this compartment, even at very early time points, as seen by the large difference in [^{3}H]insulin radioactivity in the plasma of normal and diabetic rats, is supported by the fact that a high dose of unlabeled insulin (20 mg) injected into rabbits slowed the subsequent rate of disappearance of labeled insulin from plasma (8). The association of insulin with its receptor in vivo appears to be much more rapid than that observed in vitro, possibly reflecting the higher receptor concentration and the physiological environment encountered in vivo (8). After receptor binding, our results would suggest that although some insulin may dissociate from target cells to reappear in the circulation, there is an increasing amount of receptor-linked degradation with time. Consequently, the increased capacity of the insulin-receptor compartment in diabetic rats results in more rapid and pronounced insulin degradation.

Insulin degradation has been shown to be mediated through binding of insulin to specific receptors as the initial step in vitro (37). The number of insulin receptors on the membrane may be an important determining factor in the rate of insulin degradation by intact tissues. Indeed, in states of hyperinsulinism, where a decreased number of receptors is found, insulin degradation by the liver is decreased despite an increase in total degrading activity found intracellularly (38). The situation in vivo, however, is more complex. In addition to saturable, insulin specific pathways of degradation (principally in the liver), there are also less specific, nonsaturable pathways (principally in the kidneys) (39). Thus, in the experiments where the receptor compartment was presaturated (Fig. 3), the tracer was still cleared at a relatively rapid rate, indicating that under such conditions, the nonsaturable pathways of degradation play a relatively important role. A final analogy to the present study is the observation that increased insulin binding by isolated skeletal muscle from streptozotocin diabetic rats is accompanied by increased degradation in vitro and that these effects are corrected by insulin therapy (5). It is important to note, however, that the present studies do not provide unequivocal evidence for the proposed relationship between insulin binding to receptors and degradation. It remains possible that insulin degradation in vivo occurs via a degradative pathway displaying similar binding kinetics to those observed for the insulin receptor. However, taken with evidence from experiments using high and low "affinity" insulins in vivo (8), as well as recent morphological studies on insulin absorption in target cells (40), the concept of receptor-mediated degradation remains the most logical mechanism.

We have observed that the streptozotocin diabetic rats required massive doses of insulin to restore glycemia and urine volume towards normal. Although insulin binding is necessary for the hormonal effect, other factors after binding may modify insulin action: the postreceptor effects. Despite the fact that the diabetic rats have an increased binding capacity for insulin, they still display a marked resistance to the hormone. This probably reflects a postreceptor defect in these animals, confirming previous observations in vitro (3) and in vivo in alloxan-diabetic dogs (41). Interestingly, since insulin is more rapidly degraded in these animals, presumably via a receptor-mediated pathway, our results suggest that insulin degradation and biological action need not necessarily be linked. The end result of increased insulin binding and consequently increased insulin degradation, combined with a postreceptor defect, is a remarkably uneconomical use of whatever insulin remains in these diabetic animals.

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