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Research Article

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Differing Effects of Antiinsulin Serum and Antiinsulin Receptor Serum on ^{123}I-insulin Metabolism in Rats

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Abstract

Anesthetized rats were treated with saline, antiinsulin receptor serum, or antiinsulin serum, and the biodistribution of high pressure liquid chromatography-purified ^{123}I-Tyr A14-insulin was studied by scintillation scanning. Time activity curves over organs of interest were calibrated by sacrificing the rats at the end of the experiment and directly determining the radioactivity in the blood, liver, and kidneys. Saline-treated rats exhibited normal insulin biodistribution. The highest concentration of ^{123}I-insulin was found in the liver, and reached 30% of total injected dose between 3 and 5 min after injection. After this peak, activity rapidly decreased with a $t_{1/2}$ of 6 min. Activity of ^{123}I-insulin in kidney showed a more gradual rise and fall and was ~15% of injected dose at its maximum.

In rats treated with antiinsulin antiserum, insulin biodistribution was markedly altered. Peak liver activity increased with increasing antibody concentration with up to 90% of injected dose appearing in the liver. In addition, there was no clearance of the liver ^{123}I-insulin over 30 min. Autoradiographic studies demonstrated that in contrast to the normal rats in which radioactivity was associated with hepatocytes, in rats passively immunized with anti-insulin serum, ^{125}I-insulin was associated primarily with the Kuppfer cells.

In contrast, antibodies to the insulin receptor markedly inhibited ^{123}I-insulin uptake by the liver. Kidney activity increased, reflecting the amount of free ^{123}I-insulin that reached this organ. This is similar to the pattern observed when insulin receptors are saturated with a high concentration of unlabeled insulin. Thus, both insulin antibodies and anti-receptor antibodies alter the distribution of insulin, but with very different patterns. The use of ^{123}I-insulin and scintillation scanning allows one to study specific alterations in insulin distribution in animal models of insulin-resistant states, and should also be useful in human disease states.

Introduction

We recently reported that the distribution of insulin can be studied in intact animals and in man using insulin labeled with ^{123}I-iodine and detection by a scintillation camera (1, 2). After intravenous injection, ^{123}I-insulin is rapidly concentrated by the liver and kidneys. The liver; but not the kidney uptake, can be inhibited by simultaneous injection of unlabeled insulin, suggesting that it is receptor-mediated. Both organs then show characteristic patterns of clearance as the insulin is degraded and iodide is excreted in the urine.

This new technique offers a unique tool to study insulin resistance syndromes. Two of the best characterized syndromes of insulin resistance are those that occur in patients who develop antibodies to insulin (3, 4) or antibodies to the insulin receptor (5, 6). Both of these syndromes present characteristic clinical features, and the antibodies that produce them have been extensively studied in vitro. Most information about the mechanisms by which these two types of antibodies produce insulin resistance, however, is based on extrapolation of the in vitro data to the in vivo situation. In the first case, insulin is presumed to be bound to circulating immunoglobulins, thus preventing its access to receptors on target cells. In the second, insulin also fails to reach receptors on target cells, but in this case, the blockade is presumably at the receptor itself. Thus, one would predict that the biodistribution of insulin in these two syndromes would have some similarities and some differences. In this report, we have studied these two models of insulin resistance in vivo using the scintillation scanning technique, and have also characterized the cellular distribution of antibody-bound insulin in liver using light microscopic autoradiography.

Methods

Materials. Purified bovine insulin (0.1 mg; Novo Industrie A. S., Copenhagen, Denmark) was labeled with 5 mCi ^{123}I-NA and the species monoiodinated on $TyrA_{14}$ was rapidly purified by high pressure liquid chromatography (1, 7). Bovine insulin was iodinated with ^{125}I in a similar manner and also purified by high pressure liquid chromatography. Antiserum to the insulin receptor (AIRS)[1] was from patient B2 and was previously well characterized (6, 8, 9). This serum has been shown to contain no detectable anti-insulin antibodies but has a high titer of anti-receptor antibodies. The anti-insulin serum (AIS) used for these experiments was prepared by P. H. Wright (Indianapolis, IN) by immunizing guinea pigs against bovine insulin and was a gift from Dr. J. S. Soeldner (Boston, MA).

Biodistribution and scintillation scanning. Fed male Sprague-Dawley rats weighing 225–275 g were anesthetized with pentobarbital (60 mg/kg i.p.) and a PE50 polyethylene tube was introduced into the jugular vein. Rats were laid in the prone position on the collimator of a scintillation camera (Searle Large Field of View; Des Plaines, IL) connected to a computer (Gamma 11; Digital Equipment Corp., Marlboro, MA) or a 400 T Maxicamera connected to a Star Computer (General Electric Medical Systems, Denmark). Rats received saline,

Parts of this work were performed when Dr. Sodoyez and Dr. Sodoyez-Goffaux were on sabbatical leave in the Children's Hospital Medical Center and the Joslin Diabetes Center, Harvard University, Boston, MA.

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1. *Abbreviations used in this paper:* AIRS, antiserum to the insulin receptor; AIS, antiinsulin serum.

AIS (150–250 μl), or AIRS (4–40 μl) 5–10 min before injection of ^{123}I-insulin (50–100 μCi). Immediately after the intravenous bolus of ^{123}I-insulin, sequential frames were recorded by the computer at a rate of one per 30 s for 30 min. At the end of the recording period, the animals were bled by heart puncture, sacrificed, and their organs were rapidly dissected. A 1-min static frame of the carcass and isolated liver, spleen, kidneys, and stomach was then recorded and used to measure the percentage of radioactivity in the liver and kidneys at 30 min.

Computer analysis of kinetic data was performed as previously described (2). Briefly, the actual injected radioactivity (microcuries) was measured by counting the activity of the ^{123}I-insulin-containing syringe before and after injection. Using the static frame taken at 30 min, the apparent radioactivity (cpm) of the isolated kidneys, liver, stomach, and remaining carcass was measured, and the sum of these values was taken as 100% of injected radioactivity. The percentage of radioactivity in the liver and kidney at 30 min was calculated. Regions of interest were defined over the right upper part of the liver and the lower pole of each kidney and time-activity curves were generated. After background subtraction, the ordinate of each curve was calibrated using the percentage of radioactivity in the corresponding organs at 30 min.

Anti-insulin and anti-receptor antibody assays. Blood samples taken at 30 min were immediately centrifuged and the serum was stored at −25°C for at least 1 wk to ensure complete decay of the ^{123}I. The sera were then analyzed for antiinsulin receptor or anti-insulin antibodies. Quantitation of the anti-insulin receptor antibody level in the rat serum was performed using the binding inhibition assay (5, 8). For this assay, serum from a control rat and from rats injected with anti-receptor serum was preincubated with IM-9 lymphocytes at 37°C for 15 min, after which ^{125}I-insulin was added and the cell suspensions were further incubated for 30 min. At the end of the incubation, cells were isolated by centrifugation and cell bound radioactivity was measured. Anti-insulin antibody level in the rat serum was measured by incubating serial dilutions of serum with ^{125}I-insulin at 4°C overnight, after which activated charcoal was added, the tubes were centrifuged, and the percentage of antibody bound radioactivity was measured in the supernate (10).

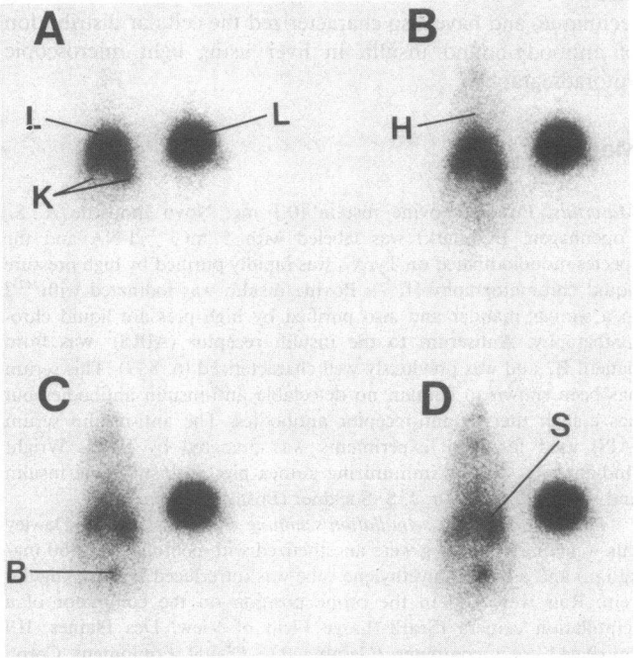

Figure 1. Scintigraphic images taken 3 (*A*), 10 (*B*), 20 (*C*), and 30 (*D*) min after injection of ^{123}I-insulin. Rats on the left of each pair were pretreated with saline, whereas those on the right received 40 μl anti-insulin serum 5 min before study. H, heart; L, liver; K, kidney; B, bladder; and S, stomach.

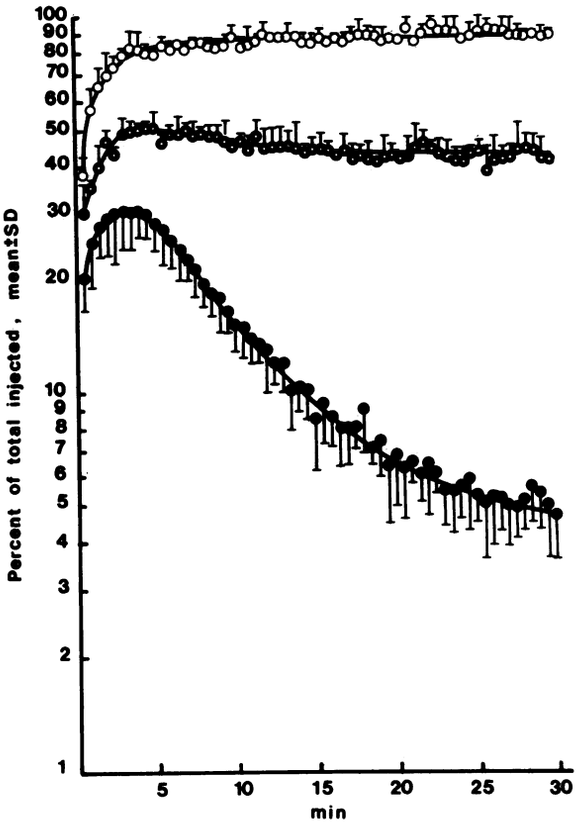

Figure 2. Effect of AIS on liver activity. Time activity profile of radioactivity in the liver of control rats or of rats pretreated with anti-insulin serum. ●, data on control rats (n = 10); ●, data on rats receiving 4 μl anti-insulin serum (n = 3); ○, data on rats receiving 40 μl anti-insulin serum (n = 3).

Autoradiography. To determine to which liver cell type radioactivity was bound, two control and two AIS-treated rats received 0.5 μCi/g ^{125}I-insulin. 9 min later, the animals were sacrificed, their livers were dissected, fixed, and sectioned, and autoradiographs were prepared as previously described (11). Photomicrographs of 1-μm thick sections were taken using a photomicroscope II (magnification of 320 on the 24 × 36 negative; Carl Zeiss, Inc., Thornwood, NY).

Data analysis. The biomedical computer program for analysis of variance of repeated measures (BMDPV) was employed to test the differences between experimental and control groups and the interaction with time (12). Criteria of homoskedasticity were satisfied by log transformation of the variables and statistical differences were determined with the Snedecor (F) test in the BMDP program (12).

Results

Effects of antiinsulin serum on biodistribution of ^{123}I-insulin. The biodistribution of insulin in control (saline-treated) rats is illustrated in Fig. 1 (left rat of each pair). 3 min after injection of ^{123}I-insulin, radioactivity was predominately concentrated by the liver and kidneys (Fig. 1 *A*, left). We have previously shown that most of this hepatic uptake is receptor-mediated and is blocked by saturation with excess unlabeled insulin (1). By contrast, renal uptake seems to be largely nonreceptor-mediated and actually increases after injection of unlabeled

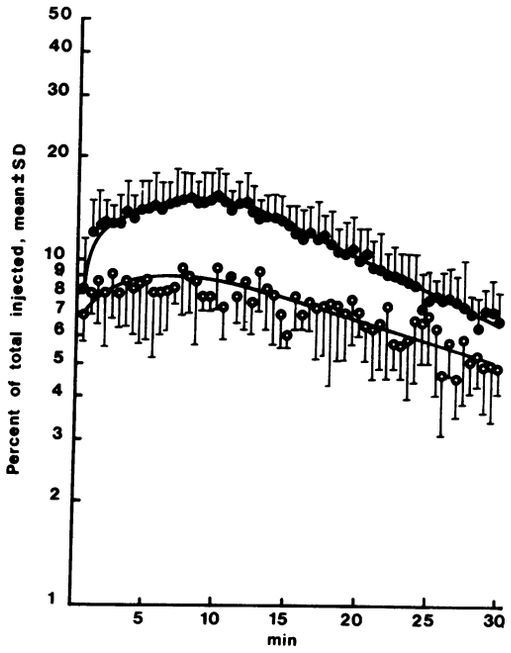

Figure 3. Effect of AIS on kidney activity. Time activity profile of ^{123}I-insulin in the kidneys of control rats (●, n = 9) and rats pretreated with 4 μl antiinsulin serum (⊙, n = 3). Kidney activity was undetectable in rats pretreated with 40 μl antiinsulin serum (○, n = 3).

insulin. At later times, liver activity decreased and by 30 min (Fig. 1 *D*, left) the pattern of distribution became similar to that of free iodide, i.e., most of the radioactivity was in the bladder and stomach. Pretreatment of the rat with antiinsulin markedly altered the distribution of serum ^{123}I-insulin (right rat of each pair). By 3 min after injection, almost all of the ^{123}I-insulin was concentrated in the liver (Fig. 1 *A*, right). This pattern remained unchanged at later times (Fig. 1 *B*, *C*, and *D*), and at no time were the kidneys, bladder, and stomach visualized.

The kinetics of hepatic uptake of ^{123}I-insulin and the effect of AIS is further demonstrated by Fig. 2. In control rats, the maximum of liver activity was 30% at 3–4 min. Past this maximum, liver activity rapidly decreased with a $t_{1/2}$ of ~6 min. After injection of 4 or 40 μl of AIS, liver activity rapidly rose to 50 or 90% of total injected, respectively. Liver activity then remained at this plateau value throughout the 30-min observation period. Both the difference in peak uptake and kinetics of disappearance were highly significant (mean of control vs. 4 μl AIS was $F = 418$, $P < 0.0001$; mean of control vs. 40 μl AIS was $F = 923$, $P < 0.0001$; interaction between groups and time were $F = 38.7$ and $F = 55.4$, respectively, both $P < 0.0001$). In contrast to the increased hepatic uptake, kidney uptake was decreased after 4 μl of AIS and abolished with 40 μl (Fig. 3) ($F = 13.7$, $P < 0.004$).

Quantitation of antibodies remaining in the serum of the rats 40 min after injection of AIS indicated that the rats injected with 40 μl AIS possessed significant titers of antibody (Fig. 4). The titer at the end of the experiment was only about 0.1% that of the original antiserum or about a third of that predicted by dilution, suggesting that much of the antibody had been cleared. In rats injected with 4 μl AIS, anti-insulin antibodies were barely detectable at 40 min after injection (Fig. 4).

In view of the marked hepatic uptake and difference in kinetics, autoradiographic studies were performed to determine the cellular localization of the insulin. In the livers of control rats, the autoradiographic grains were predominantly associated with the hepatocytes (Fig. 5 *A*). By contrast, in the rats that received AIS, the grains were almost exclusively localized over Kuppfer cells (Fig. 5 *B*).

Effect of antiinsulin receptor serum on the biodistribution of ^{123}I-insulin. Fig. 6 illustrates the effect of AIRS on ^{123}I-insulin biodistribution. In each panel, the rat on the left side of the field was treated with saline and exhibits the same pattern of activity distribution as in nontreated controls, whereas that on the right was pretreated with 250 μl of anti-receptor serum B2 (AIRS). In the rat pretreated with 250 μl AIRS, the liver was not visible on the 3-min image but the kidneys were conspicuous (Fig. 6 *A*, right). At later times, the kidneys remained visible and evidence for free iodide appearance, i.e., activity in the stomach and bladder, became prominent, but little activity appeared in the liver.

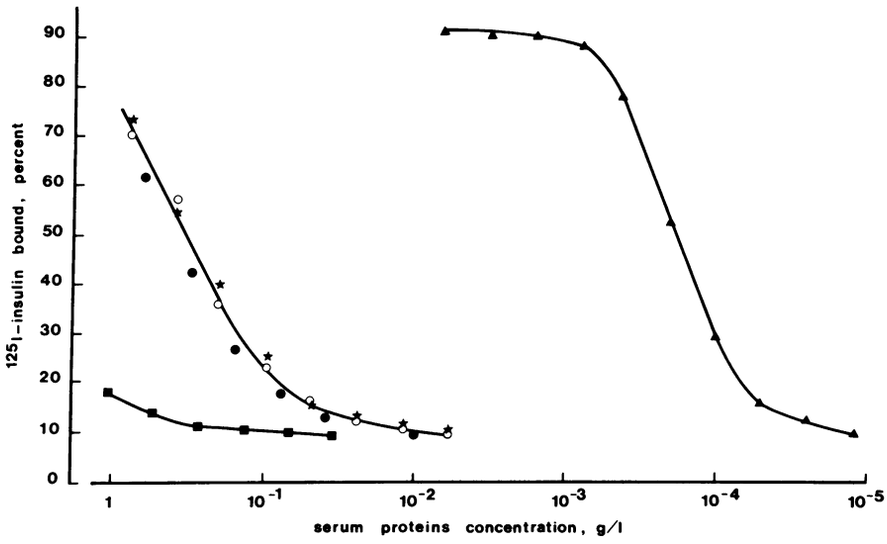

Figure 4. Titration curve of serial dilutions of guinea pig antiinsulin serum (—▲—) or of serum obtained from three rats pretreated with 40 μl antiinsulin serum (—●—, —○—, —★—) or from one rat pretreated with 4 μl antiinsulin serum (—■—). In each case, the sera were lyophilized before assay and the results were standardized by measuring plasma protein concentration.

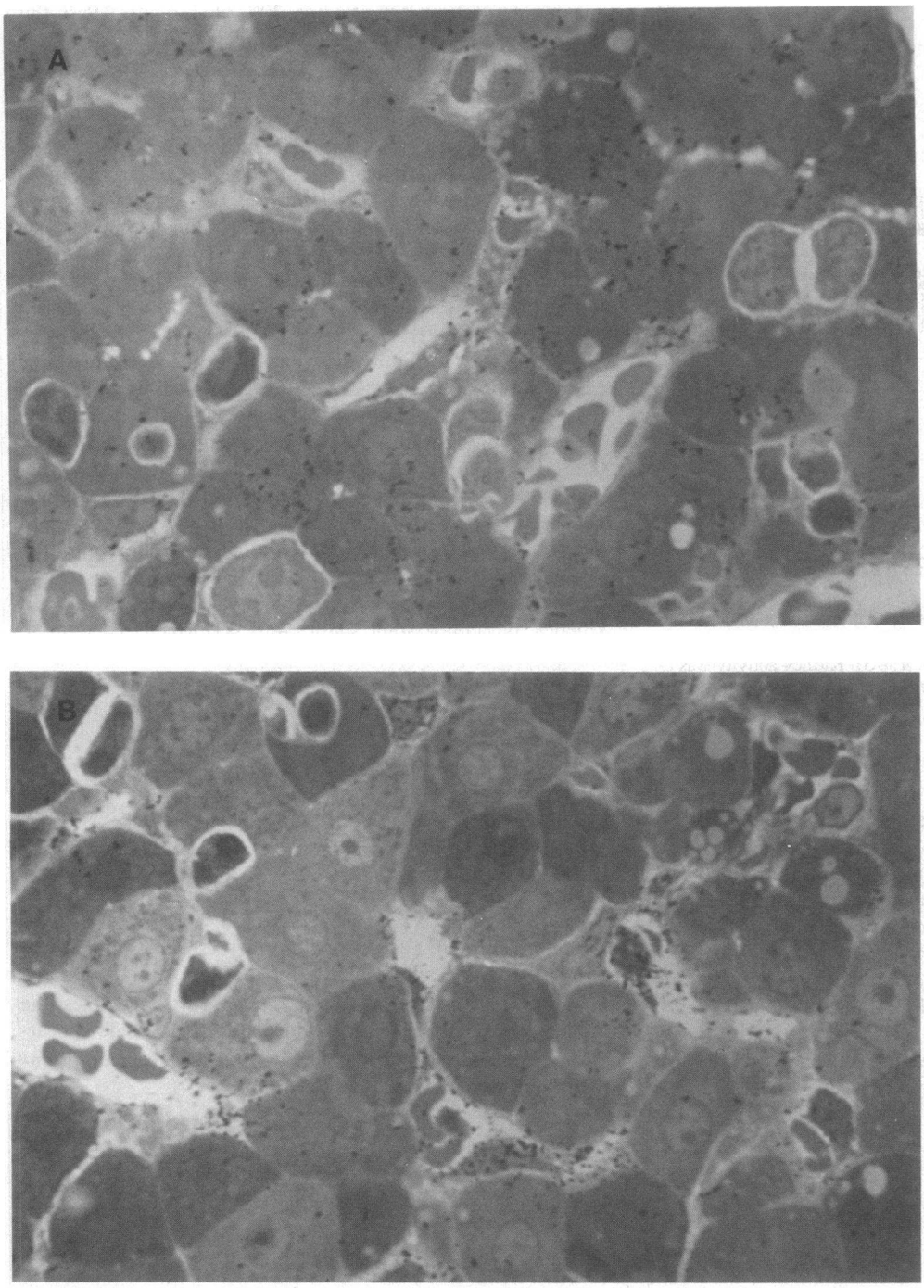

Figure 5. Autoradiograph of livers taken 9 min after ^{125}I-insulin injection (*A*) into a control or (*B*) into a rat pretreated with 40 μl antiinsulin serum. In *A*, silver grains are associated with the hepatocytes, whereas in *B* they are associated primarily with Kuppfer cells.

This effect of AIRS was confirmed by examining liver time activity curves (Fig. 7). In the presence of 250 μl AIRS, liver activity was reduced to 9% at 3 min compared with 30% in control rats ($F = 32.8$, $P = 0.0003$). Injection of a lower dose of AIRS (100 μl) had less of an effect on liver ^{123}I-insulin uptake and the liver activity profile was indistinguishable from that of control rats ($F = 2.85$, $P = 0.12$ for comparison of group means). By contrast, the two doses of AIRS increased kidney activity in a dose-dependent manner (Fig. 8) ($F = 6.6$, $P = 0.03$ for comparison of control and 100 μl AIRS pretreated rats; $F = 23.5$ $P = 0.0009$ for comparison of control and 250 μl AIRS pretreated rats). The time activity profiles for the kidney of AIRS-treated rats remained parallel to that of control rats ($F = 0.73$ and 0.95 when comparing control and 100 μl AIRS or 250 μl AIRS pretreated rats).

The actual titers of antibodies to the insulin receptor in the serum of rats pretreated with serum of patient B2 were determined by titrating a serum sample taken immediately after sacrifice. As shown by Fig. 9, the serum from rats injected with 250 μl of antiserum inhibited ^{125}I-insulin binding to IM-9 lymphocytes in vitro, whereas the serum of a rat injected with 100 μl antiserum was not significantly different from control. The small effect of the control serum compared with our previous studies is probably the result of the modified

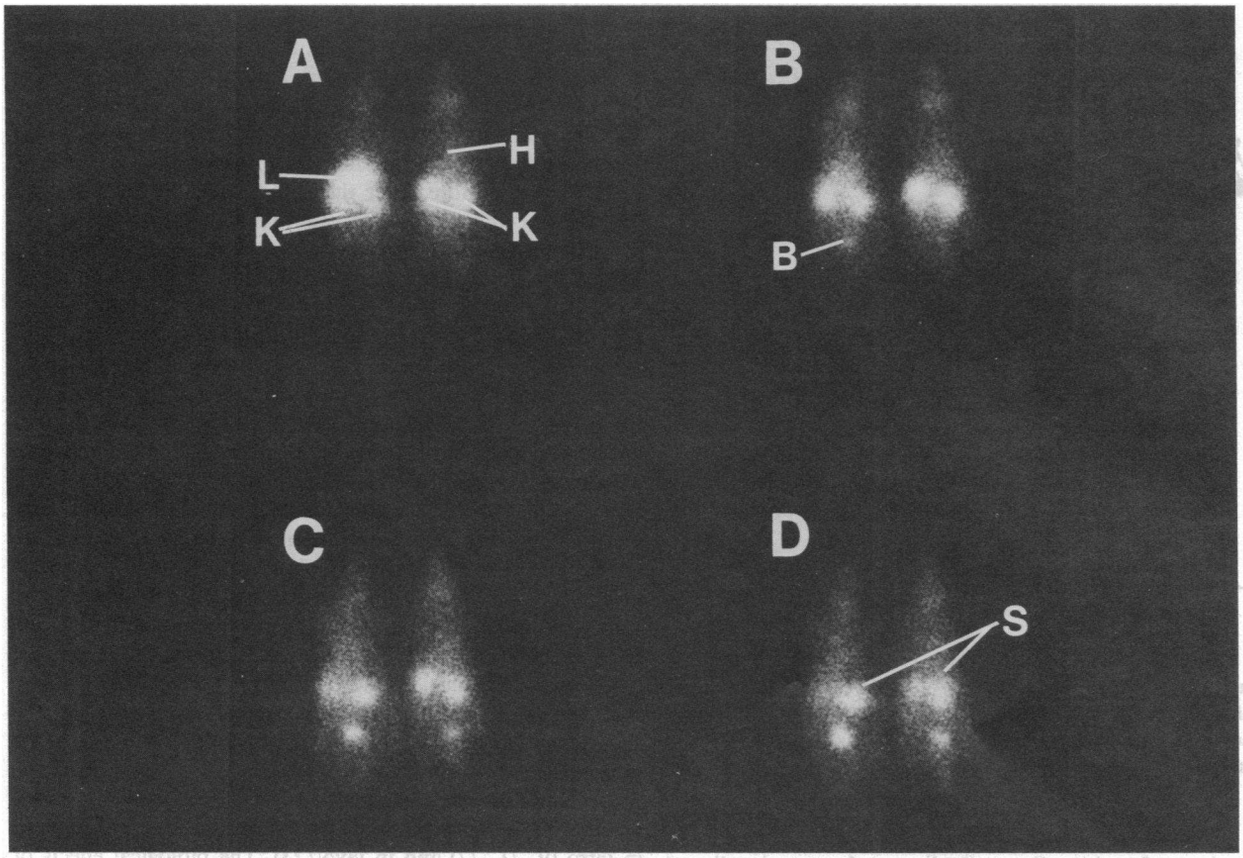

Figure 6. Polaroid images taken 3 (*A*), 10 (*B*), 20 (*C*), and 30 (*D*) min after ^{123}I-insulin injection. Rats were pretreated with saline (left rat in each pair) or 250 μl antiinsulin receptor serum (right rat in each pair) 10 min before study. Note in *A* that 3 min after ^{123}I-insulin injection, the liver of the antiinsulin receptor serum injected rat is barely visible. Abbreviations are as in the legend to Fig. 1.

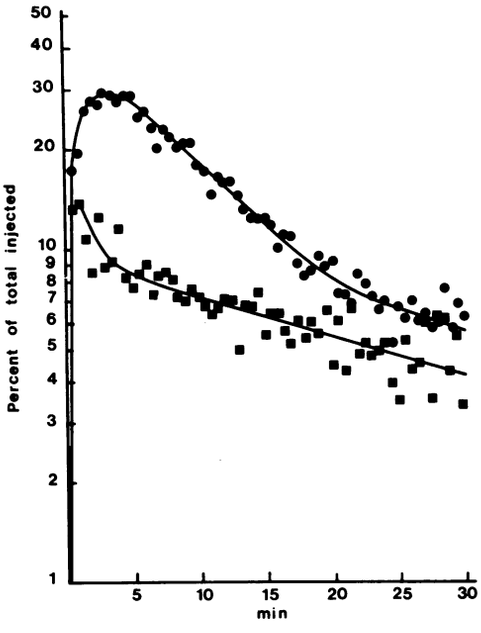

Figure 7. Effect of AIRS on liver activity. Time activity profile of ^{123}I-insulin in the livers of rats pretreated with 100 (●) or 250 (■) μl antiinsulin receptor serum (n = 2 in each group). Note that in the presence of 100 μl serum, the liver curve is indistinguishable from that of saline pretreated rats (compare with Fig. 2).

reaction conditions (37°C, in the continued presence of serum) that were designed to more closely mimic the in vivo situation.

Discussion

Little is known about the distribution of insulin in intact animals and even less in insulin-resistant states. Metabolic distribution of insulin has been estimated primarily by studies of the disappearance of the hormone from plasma or by counting organ-associated radioactivity after sacrifice of animals injected with ^{125}I-, ^{131}I-, or ^{3}H-insulin (13–15). With the use of ^{123}I, it is now possible to study directly the kinetics and biodistribution of insulin in intact animals using scintillation scanning. Our results clearly indicate that the liver plays a key role in the clearance of plasma insulin. The quantitative importance of the liver in plasma insulin disposal is evidenced by the fact that even after a peripheral venous injection, maximum liver activity was 30% of total between 3 and 5 min. As will be reported in more detail in another study, this figure underestimates the liver clearance of endogenous insulin that is secreted into the portal circulation and may have an uptake in excess of 70% of injected dose (16). The biological half-life of radioactivity in the liver using this method is short, 6 min, a figure that agrees with a previous estimate obtained by sequential measure of actual liver ^{125}I-insulin (17). As recently noted, there is thus a marked discrepancy between the kinetics of insulin degradation in the liver and that of deactivation of insulin effects in the same organ (18).

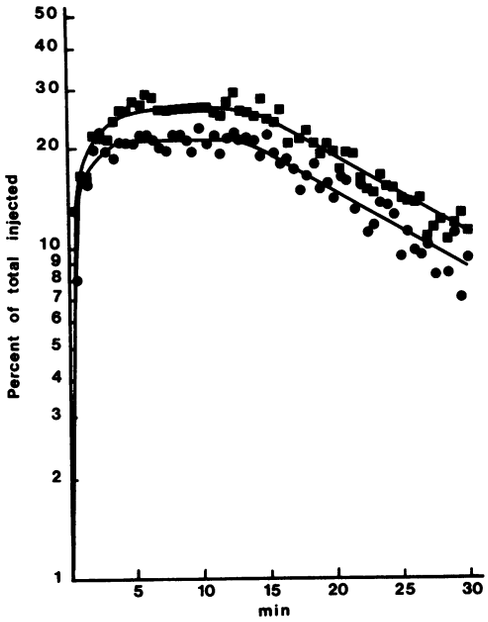

Figure 8. Effect of AIRS on kidney activity. Profile of kidney radioactivity in rats previously treated with either 100 (n = 2, ●) or 250 (n = 2, ■) μl antiinsulin receptor serum. Comparison with Fig. 3 shows that kidney uptake of radioactivity was enhanced by AIRS in a dose-related manner.

High titers of anti-insulin antibodies produce an insulin-resistant state; however, the exact mechanism is unclear. Antibodies may bind insulin and thus prevent access of insulin

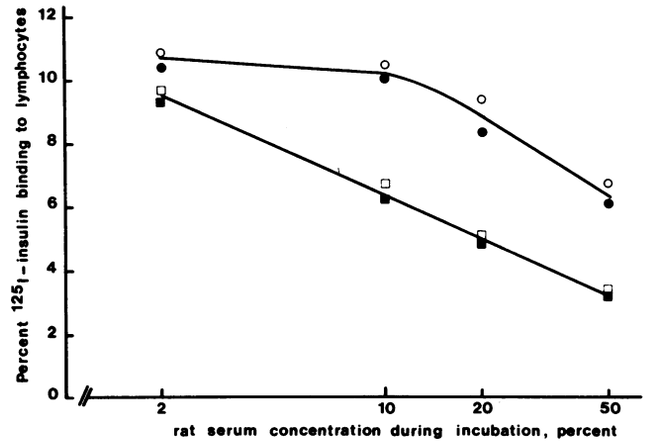

Figure 9. Inhibition of ^{125}I-insulin binding to IM-9 lymphocytes by serial dilutions of serum samples obtained from a control rat (●) and rats pretreated with antiinsulin receptor serum (○, 100 μl AIRS; □ and ■, 250 μl AIRS). The serum samples were drawn by heart puncture immediately at the end of the 30-min recording period, i.e., ±40 min after AIRS injection, and the titer of antibody was determined by the binding inhibition assay described in Methods. In the absence of any serum, cells bound 11% of the tracer. Nonspecific binding was 4%. The inhibition of ^{125}I-insulin binding by the serum of 250 μl AIRS-treated rats was not due to hyperinsulinemia, since plasma immunoreactive insulin concentration was similar in control and experimental rats.

to its target tissues (3, 4). Alternatively, the immune complexes may be cleared without releasing their insulin, thus creating a new pathway of insulin loss (19). In this study, we find that antibody-bound insulin rapidly disappears from the circulation. In these passively immunized rats, ^{123}I-insulin was taken up by the liver in increasing amounts in proportion to the dose of AIS. Furthermore, after the first 5 min, liver activity did not decrease as in control rats, but remained constant throughout the remainder of the experiment. The most likely explanation for these two observations is that ^{123}I-insulin-immune complexes, and not free ^{123}I-insulin, reacted with the liver. As recently shown by Maron et al. (19a) and Taylor et al. (20), when insulin binds to its receptors, some of the hormone remains accessible to anti-insulin antibody but the majority of the insulin molecule is unavailable for binding to anti-insulin antibodies, probably because most of the insulin molecule is sequestered in the receptor. Conversely, it may be inferred that insulin bound to most antibodies is unavailable for receptor binding. This is consistent with our finding that the ^{123}I-insulin immune complexes bind poorly to hepatocytes, and autoradiographs of the liver of passively immunized rats show that under these conditions radioactivity is almost exclusively associated with the Kuppfer cells. Thus, the trigger for ^{123}I-insulin clearance by the liver is no longer the hormone itself binding to its receptors on hepatocytes, but probably the Fc fragment of the insulin-immune complexes binding to Fc receptors on macrophages.

Anti-insulin receptor antibodies have been shown to block insulin binding in vitro (5, 9) and to alter insulin action both in vitro (9, 21, 22) and in vivo (23). The biological effects of the antibody are complex, mimicking insulin action acutely (9, 21, 22, 23), and inducing a state of insulin resistance after more prolonged exposure (21, 23). The serum of patient B2 used in these experiments has been well characterized and is shown to have a high titer of anti-receptor antibodies that react specifically with insulin receptors on a wide variety of cells with little or no anti-insulin antibodies (5, 6, 9). After pretreatment with 250 μl of this serum, liver uptake of ^{123}I-insulin was almost completely abolished in the rat. With this concentration of antiserum, free antibodies were still detectable in the rat serum at the end of the experiment, indicating that antibodies were in excess with regard to receptors. The lower dose of AIRS had no visible effect on ^{123}I-insulin uptake by the liver, suggesting that specific receptors in this organ are in excess with regard to circulating insulin.

The kidneys also play an important role in clearance of plasma insulin, although by a more complex mechanism (24). The hormone is filtered through the glomerular membrane and reabsorbed by the brush border of the proximal tubular cells (25–28). It is not yet clear whether this reabsorption process is receptor mediated or not (29–32), although our results suggest the latter is the case since this process is not saturable. A smaller portion of insulin is cleared by a post-glomerular mechanism involving the contraluminal aspects of the tubular cells. There is evidence that this peritubular clearance is receptor mediated (32–35). In our previous studies, we found that saturation of the receptor compartment abolished ^{123}I-insulin uptake by the liver and increased tracer uptake by the kidney (1). A similar pattern was observed in AIRS-treated rats. In these, the lower dose of AIRS and to a greater extent the higher dose of AIRS increased the kidney uptake of ^{123}I-insulin. These two observations can be explained if blockade

of the receptor compartment of the liver abolishes insulin clearance by this organ, and as a consequence the ^{123}I-insulin load to the kidneys is increased. This mechanism more than compensates for a possible decrease of peritubular clearance so that the end result is an enhanced kidney uptake of radioactivity.

The fact that kidney uptake was decreased or abolished by antiinsulin serum supports the contention that only free ^{123}I-insulin is cleared by the contraluminal aspect of the tubular cells and by glomerular filtration. As previously discussed, antibody-bound insulin is not available for receptor binding and the immune complexes are too large to be filtered. Thus, in all conditions examined so far, presence of an excess of unlabeled insulin, of AIS, or of AIRS, total kidney radioactivity reflects the amount of circulating free ^{123}I-insulin.

Whereas the rats treated with AIRS seem to be a coherent model of human type B insulin resistance syndrome (5, 6), rats passively immunized with AIS do not mimic all disorders of insulin metabolism in insulin-immunized diabetic patients. Indeed, in preliminary studies, we have observed that serum taken from patients with high anti-insulin antibody level and insulin resistance may have varying effects on insulin clearance. Some antibodies have an insulin 'scavenging' effect similar to that observed in passively immunized rats, whereas antibodies taken from other patients primarily retard the clearance of insulin from the plasma (27). Thus, it seems that there is more than one type of antibody to insulin and these affect insulin bioavailability and metabolism differently, perhaps depending on their affinity, the epitope(s) of insulin with which they react, and perhaps also depending on the immunoglobulin subclass (Sodoyez, J. C., E. R. Arquilla, B. McDougall, C. J. De Vos, R. Von Frenckell, and F. Sodoyez-Goffaux. 198–. In vivo scintigraphic distribution of complexes of antiinsulin immunoglobulin subclasses and ^{123}I-insulin, manuscript submitted for publication).

In summary, using the technique of scintillation scanning with ^{123}I-insulin, it is possible to measure simultaneously the relative importance of insulin clearance by the kidney and insulin binding to its receptors and receptor-mediated degradation in important insulin target tissues such as the liver in normal animals and insulin resistant states. This technique can therefore provide new insights into the mechanism of insulin resistance in man and other animals.

Acknowledgment

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