Insulin-Like Growth Factor I Resistance in Peripheral Tissue but not in Liver in Streptozotocin-Induced Diabetic Rats

MAKOTO TOMINAGA, KEICHI YAMATANI, MASAKI IGARASHI, HIDEYUKI EGUCHI, MAKOTO DAIMON, AKIRA SEKIKAWA, AND HIDEO SASAKI

Abstract. The metabolic effect of recombinant human insulin-like growth factor I (IGF-I) was investigated by the glucose clamp technique in normal rats and streptozotocin-induced diabetic rats, a model of insulin-dependent diabetes mellitus (IDDM), and compared with that of insulin. Glucose uptake by peripheral tissues was stimulated by intravenous administration of IGF-I at rates of from 0.369 to 3.690 nmol/kg/min in a dose dependent manner, with a potency of 1/52 that of insulin estimated on the basis of the ED$_{50}$ molar ratio in normal rats. In streptozotocin-induced diabetic rats, the maximum effects of IGF-I and insulin were reduced to 72% and 70% of those in normal rats, respectively, indicating the presence of both IGF-I and insulin resistance. Hepatic glucose output in normal rats was suppressed by IGF-I in a dose dependent manner with a weaker potency of 1/99 that of insulin assessed on the basis of the ED$_{50}$ values. In streptozotocin-induced diabetic rats, a dose-response curve of the suppressive effect of insulin on hepatic glucose output shifted to the right, indicating the presence of hepatic insulin resistance, but a leftward shifting of the suppressive effect of IGF-I on hepatic glucose output was observed. We concluded that the IGF-I effect on peripheral tissue was decreased but that on the liver was rather increased in streptozotocin-induced diabetic rats, in contrast to the resistance of both peripheral tissues and liver to insulin.

Key words: IGF-I, Streptozotocin, Insulin resistance, IGF-I resistance, Glucose clamp

THE ADMINISTRATION of insulin-like growth factor I (IGF-I) has been shown to lower blood glucose levels in healthy subjects [1]. Even in an insulin resistant state characterized by insulin receptor abnormality in which the administration of insulin could not decrease blood glucose levels, IGF-I decreased blood glucose levels probably through intact IGF-I receptors [2]. However, it is still not certain whether IGF-I is also effective on more common types of insulin resistance, caused by abnormality of post-binding signal transduction process, not by abnormality of the insulin receptor itself. Such abnormality is known to occur both in insulin-dependent diabetes mellitus (IDDM) and non-insulin dependent diabetes mellitus (NIDDM) [3]. In the present study, we investigated the metabolic effect of IGF-I in streptozotocin-induced diabetic rats, a model of IDDM, by means of the glucose clamp technique and compared it to that of insulin.
Materials and Methods

Streptozotocin-induced diabetic rats and treatments

Male Wistar rats, weighing 252 ± 17 g (mean ± SD) and aged 100 ± 11 days were purchased from Funabashi Farm (Chiba, Japan) and kept with free access to a standard laboratory chow and water, in a room of our university animal ward with controlled lighting (lights on from 0600 to 1800 h) and controlled temperature (22 °C). The rats were randomly divided into two groups, a streptozotocin-induced diabetic group (n=36) and a normal control group (n=42). Into rats of the streptozotocin group, 50 mg/kg of streptozotocin (Sigma, St. Louis, MO, USA) dissolved in 0.05 M citrate buffer (pH 4.5) was injected into the femoral vein. The diabetic rats were allowed to recover for 2 days after the streptozotocin injection, then a minimum dose (1–2 u/day) of Ultralente insulin (Novo, Denmark) was subcutaneously injected between 1400 h and 1600 h everyday for 2 weeks. Specimens of urine from these rats were negative for keton bodies but contained a lot of glucose. Twenty to 24 h before the clamp study, 1 unit of Ultralente insulin was injected and food was withheld.

Normal control rats were also subjected to the glucose clamp study after 20 to 24 h fasting.

Glucose clamp technique

The glucose clamp technique, originally introduced by Andres [4] and DeFronzo [5], was employed with a slight modification, described earlier [6, 7]. Under 0.35 g/kg ip chloral hydrate anesthesia (Nakarai Chemical, Kyoto, Japan), three Silastic catheters (Dow-Corning Corp., Midland, MI, USA) were cannulated into the left and right femoral veins, for the infusion of 3H-3-glucose (New England Nuclear, Boston, MA, USA), for 20% glucose, and for human recombinant IGF-I (FK780), provided by Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan [8], or human insulin (Humalin®, Shionogi Pharmacy Co. Ltd., Osaka, Japan). A double lumen catheter was inserted into the right jugular vein for blood sampling by Glucose Monitor, GM-1320 (Kyoto Daiichi Scientific Equipment Co., Ltd., Kyoto, Japan). Another Silastic catheter was cannulated into the left carotid artery for blood sampling to determine the specific activity of 3H-3-glucose and hormone levels.

Under the same anesthesia, after a 5 μCi bolus injection, 3H-3-glucose was continuously infused at the rate of 0.05 μCi/min with a peristaltic roller pump (Minipuls 2, Gilson, France) until the entire clamp ended. Fifty-five and 60 min after the beginning of 3H-3-glucose infusion, 0.1 ml blood samples were taken to determine the glucose disappearance rate in the basal steady state.

IGF-I and insulin, dissolved in saline containing 0.25% BSA (Intergen Co., Purchase, NY, USA), were infused at rates of 0.369, 0.923, 3.690 nmol/kg/min and 0.5 (0.003), 2 (0.012), 5 (0.031), 20 (0.123) mU/kg/min (nmol/kg/min), respectively, into both streptozotocin-induced diabetic rats and normal control rats, with an infusion pump, STC-521 (Terumo, Tokyo, Japan). The glucose clamp study of the streptozotocin-induced diabetic rats with the lowest dose of insulin (0.5 mU/kg/min) was not done, because the blood glucose level did not decrease to 140 mg/dl with such a low dose of insulin. In normal control rats, to maintain the blood glucose level at the fasting level, 139 ± 17 mg/dl, a variable rate of infusion of 20% nontritiated glucose was given through the right femoral vein, with a peristaltic roller pump, according to the procedure as described previously in detail [7]. In streptozotocin-induced diabetic rats, because of hyperglycemia, 347 ± 80 mg/dl, the infusion of 20% glucose was postponed until the blood glucose level decreased to 140 mg/dl. To maintain euglycemia in both groups, the infusion of 20% glucose was continued for 60 min. At 55 min and 60 min after the 20% glucose infusion started, 0.1 ml blood samples were taken to determine specific activity of 3H-3-glucose, and another 3 to 5 ml blood was also taken for determination of plasma hormone level at the end of the clamp.

Measurement and calculation

Serum, sampled at 55 and 60 min during the basal period before IGF-I and insulin infusion and also at 55 and 60 min of the clamp, was mixed with ZnSO₄ and Ba(OH)₂ and centrifuged. Specific activity of 3H-3-glucose in the deproteinized and dried supernatant was measured with a liquid-scintillation counter. Glucose disappearance (Gd) during both the basal and the clamp steady state was calculated by Steele’s equation [9]. During the basal state, basal hepatic glucose output
(HGO\textsubscript{basal}) was estimated to be the same as the basal Gd. The mean of every 2 min glucose infusion rate for the final 20 min of the clamp was estimated as the clamp parameter, GIR. HGO during clamp (HGO\textsubscript{clamp}) was determined by subtracting GIR from clamp Gd based on the assumption that HGO=Gd−GIR. When the HGO\textsubscript{clamp} indicated a minus value, HGO\textsubscript{clamp} was regarded as zero, and an insulin-like effect on the peripheral tissue, i.e., glucose uptake (Gu) was estimated to be the same as GIR. When the HGO\textsubscript{clamp} indicated a positive value, Gu was estimated to be equal to Gd. Percent suppression of HGO by IGF-I and insulin was calculated according to Moxley III et al. [10] as follows: % suppression of HGO=(1−HGO\textsubscript{clamp}/HGO\textsubscript{basal})×100.

IGF-I levels in the blood sampled at the end of the clamp were determined by specific radioimmunoassay using a human recombinant IGF-I as the standard [11]. Insulin levels were also determined by radioimmunoassay [12].

Data are shown as the mean ± SD, and ED\textsubscript{50} and ED\textsubscript{85} were assessed by the Probit method of SAS program with a personal computer.

Results

Effect of IGF-I and insulin on glucose uptake by peripheral tissue

IGF-I and insulin stimulated glucose uptake by peripheral tissue during the glucose clamp steady state in a dose-dependent manner in normal rats, as shown in Fig. 1A. At the highest dose of IGF-I and insulin used, glucose uptake was similarly enhanced to 24.81 ± 1.91 mg/kg/min and 24.59 ± 3.65 mg/kg/min, respectively. Taking these values as 100%, ED\textsubscript{50} of IGF-I was 0.782 nmol/kg/min and that of insulin was 0.015 nmol/kg/min, as shown in Table 1. Thus, the molar ratio of IGF-I to insulin was 1/52.

In streptozotocin-induced diabetic rats, the dose-response curves of both IGF-I and insulin shifted rightward as shown in Fig. 1A. The maximum

![Figure 1](image_url)
effects of IGF-I and insulin on these diabetic rats similarly decreased to 72% and 70% of those on normal rats, respectively, as shown in Table 2.

During the clamp at the highest dose of IGF-I, peripheral levels of IGF-I in normal rats and in streptozotocin-induced diabetic rats were 815.9 ± 227.5 nmol/l (6247 ± 1742 µg/ml, expressed as conventional units) and 599.5 ± 224.4 nmol/l (4590 ± 1718 µg/ml), respectively, without any difference, as shown in Fig. 1B. Insulin levels during the clamp at the highest dose of Insulin were 15.6 ± 7.2 nmol/l (2538 ± 1175 µU/ml) and 17.3 ± 10.5 nmol/l (2817 ± 1704 µU/ml), in normal rats and streptozotocin-induced diabetic rats, respectively, likewise not different from each other.

**Suppressive effect of IGF-I and insulin on hepatic glucose output**

A dose-dependent suppressive effect of IGF-I and insulin on hepatic glucose output was observed, as shown in Fig. 2A. ED$_{50}$ values in normal rats produced by IGF-I and insulin were 0.594 nmol/kg/min and 0.006 nmol/kg/min, respectively, as shown in Table 3. The potency of IGF-I was 1/99 that of insulin on a molar basis, indicating that the liver is less sensitive to IGF-I than peripheral tissue in normal rats.

**Table 2. Maximum effect of insulin and IGF-I on glucose uptake (mg/kg/min)**

<table>
<thead>
<tr>
<th></th>
<th>Normal rats</th>
<th>STZ-DM rats</th>
<th>STZ/Normal</th>
</tr>
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<tbody>
<tr>
<td>Insulin</td>
<td>24.59 ± 3.65</td>
<td>17.32 ± 3.82</td>
<td>0.70</td>
</tr>
<tr>
<td>IGF-I</td>
<td>24.81 ± 1.91</td>
<td>17.82 ± 3.71</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Abbreviation: same as in Table 1.

**Table 3. ED$_{50}$ and ED$_{85}$ of percent suppression of hepatic glucose output by insulin and IGF-I (nmol/kg/min)**

<table>
<thead>
<tr>
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<th>Normal rats</th>
<th>STZ-DM rats</th>
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<tbody>
<tr>
<td>ED$_{50}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>0.006</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>IGF-I</td>
<td>0.594</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ratio (Insulin/IGF-I)</td>
<td>1/99</td>
<td>–</td>
<td>–</td>
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<tbody>
<tr>
<td>ED$_{85}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>0.010</td>
<td>0.058</td>
<td>5.80</td>
</tr>
<tr>
<td>IGF-I</td>
<td>0.791</td>
<td>0.398</td>
<td>0.50</td>
</tr>
<tr>
<td>Ratio (Insulin/IGF-I)</td>
<td>1/79</td>
<td>1/24</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviation: same as in Table 1.
On the other hand, in streptozotocin-induced diabetic rats, the presence of hepatic insulin resistance was suggested by the rightward shifting of the dose-response curve by insulin as shown in Fig. 2A. However, % suppression of hepatic glucose output with the lowest dose of IGF-I (0.369 nmol/kg/min) was 80.6 ± 22.3 % in streptozotocin-induced diabetic rats, in contrast to only 4.2 ± 24.7 % in normal rats. In other words, a leftward shift occurred. Comparison of values for $ED_{50}$ of IGF-I and insulin in streptozotocin-induced diabetic rats was impossible, because a lower dose of IGF-I or insulin than shown here did not decrease the blood glucose levels to 140 mg/dl. Instead of $ED_{50}$, $ED_{85}$ values were computed, as shown in Table 3. The ratio of $ED_{85}$ of insulin in normal rats to streptozotocin-induced diabetic rats was 5.80, which meant that about a six times higher dose of insulin was needed to obtain an equivalent effect. In contrast, the ratio in the IGF-I effect was reduced to 0.50, suggesting rather a hypersensitivity of the liver to IGF-I in streptozotocin-induced diabetic rats.

**Discussion**

IGF-I has come to be known to have a glucose-lowering effect in healthy subjects and normal animals, soon after IGF-I was discovered [1]. However, the mechanism of the glucose-lowering effect was not fully understood, because of the limited source of IGF-I which had been made from pooled plasma. Whether IGF-I action is mediated by the increased uptake of glucose into the peripheral tissues or by the suppressed glucose output from the liver was not known. About 10 years ago, IGF-I was produced by applying DNA technology [8], and the problem of the limited source of IGF-I was solved.

In normal rats, higher responsiveness of peripheral tissues than the liver to IGF-I was reported by Moxley III et al. [10] with the glucose clamp technique, similar to our present study on normal rats in which the suppressive effect of IGF-I on hepatic glucose output was much less than that of insulin. In depancreatized but supplementary insulin infused dogs, a higher IGF-I/insulin potency ratio in the peripheral tissue than in the liver was also reported [13]. These difference between potency ratios (IGF-I/insulin) of metabolic effect on peripheral tissues and the liver can most likely be explained by the difference between numbers of own receptors of IGF-I and insulin in peripheral tissues and the liver. In other words, the metabolic effect of IGF-I is mediated through its own receptors, not through insulin receptors. This absence of a cross-reaction was confirmed by in vitro studies [14]. Since DiGirolamo et al. reported that adipose tissues have virtually no IGF-I receptors [15], the main peripheral organ responsible for IGF-I action is thought to be the muscles. There have been reported to be small number of IGF-I receptors in the liver [16]. However, against this, Venkatesan et al. suggested that the lesser indirect pathway-effect of IGF-I on the liver, i.e. the less potent effect of IGF-I on decreasing circulating free fatty acids from muscle and adipose tissue as compared to insulin, could be responsible for the weaker metabolic effect of IGF-I on the liver [17].

In any case, IGF-I could certainly decrease the plasma glucose levels through intact IGF-I receptors, even in the insulin resistant state without any normal insulin receptor such as Mendenhall’s syndrome [2]. If no IGF-I resistance were found in the insulin resistant state in both types of diabetes mellitus, IDDM and NIDDM in which a postreceptor defect is a common feature, IGF-I could be given as a clinical challenge in order to decrease the blood glucose levels.

The present study to investigate insulin resistance of IDDM, not NIDDM, clearly showed that peripheral tissues were resistant to both insulin and IGF-I. The maximum effects of insulin and IGF-I were identically decreased to about 70% of that of normal rats. This result was in accord with an earlier report by Jacob et al. [18] on spontaneously diabetic BB/w rats, although controversial results of no IGF-I resistance in partially pancreatectomized rats were reported by Rosetti et al. [19]. Sowell et al. [20] reported the interesting observation that rat skeletal muscle treated with phenylarsine oxide (PAO), an agent believed to block insulin stimulated glucose transport at a postreceptor level, was resistant also to IGF-I. Therefore, the same, or very similar, postreceptor pathway in both insulin and IGF-I receptors is most likely present, and this common postreceptor pathway would be identically affected in IDDM model animals. In a NIDDM model animal ob/ob mice, and in human NIDDM, a loss of sensitivity of glucose uptake by muscle to both insulin and IGF-I...
was also shown by Cascieri et al. [21], and by Dohm et al. [22], respectively.

However, the suppressive effects of insulin and IGF-I on hepatic glucose output were completely different in streptozotocin-induced diabetic rats and normal rats. In the normal rats, the ratio of ED85 of potency of insulin to IGF-I on a molar basis was 1/79. In contrast, the ratio was increased to 1/24 in streptozotocin-diabetic rats, in agreement with the earlier study by Jacob et al. [18] that in BB/w rats IGF-I infusion showed more suppression of hepatic glucose production than non-diabetic rats. The reason for the rather increased sensitivity of hepatic glucose output to IGF-I is not clearly explained. Because reduced levels of both plasma IGF-I and IGF-I binding proteins are well known in malnutrition, including severe diabetes mellitus [23, 24], our speculation, and nothing more, is that the occurrence of up-regulation of IGF-I receptors in the liver could explain the hypersensitivity of liver to IGF-I. Our speculation is supported by the observation of Scott et al., that the reduced number of IGF-I receptors of cultured hepatocytes from diabetic rats was not restored by GH administration but by insulin treatment [25].

But even our surmise cannot explain all the facts. First of all, it is based on the assumption that there are two different routes through which the post-binding signal are transduced in the liver between insulin and IGF-I, in spite of the abovementioned conclusion that almost the same post-binding pathway is present in the muscle. Secondly, the IGF-I effect on the liver in a NIDDM model animal was reported to be attenuated [26], although IGF-I binding proteins were also low in NIDDM as well as IDDM [27]. These questions remain to be elucidated in the future.

By the way, with respect to the clinical implications of IGF-I as a treatment for insulin resistance, this hypersensitivity of the liver to IGF-I in spite of resistance to insulin is fascinating. However, not only might the possible proliferating effect of IGF-I on vessel walls be adverse for patients with diabetes mellitus, but the mechanism of the rather increased sensitivity of hepatic glucose output to IGF-I should be elucidated before initiating a clinical challenge.

References


