Note

Starvation-increased Insulin-dependent Tyrosine Phosphorylation of the 195-kDa Protein in Intact Rat Liver

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Insulin stimulates tyrosine phosphorylation of 175–195-kDa proteins including insulin receptor substrate-1 (IRS-1) in various tissues and cell types. In intact rat livers, starvation increased the insulin-dependent tyrosine phosphorylation of the insulin receptor and IRS-1 as has been described by others. Surprisingly, starvation greatly increased the tyrosine phosphorylation of the 195-kDa protein induced by insulin, indicating that this protein may be a new substrate of the insulin receptor kinase. The marked increase in tyrosine phosphorylation of the 195-kDa protein may have a physiological role in signal transmission in response to insulin under starvation conditions.

Key words: insulin; tyrosine phosphorylation; insulin receptor; insulin receptor substrate-1 (IRS-1); rat liver

Insulin has a variety of biological effects including rapid metabolic and growth-promoting actions. Insulin binding to the insulin receptor z-subunit activates the intrinsic tyrosine kinase of its b-subunit.1 Activation of this kinase leads to tyrosine phosphorylation of intracellular substrates, including insulin receptor substrate-1 (IRS-1), insulin receptor substrate-2 (IRS-2), and Shc.3–5 These intracellular events are believed to be essential in leading to insulin’s final biological activities. Some researchers have shown that insulin infusion to the portal vein changes the tyrosine phosphorylation of the insulin receptor b-subunit and IRS-1 in liver and muscle of intact animals, and activates the insulin-dependent signaling cascade under various physiological conditions.6–10 In this study, we investigated the effect of starvation on changes in tyrosine phosphorylation of intracellular proteins induced by insulin in intact liver and found that tyrosine phosphorylation of a 195-kDa protein was increased under this condition.

Male Wistar rats (6 weeks old) were fed standard rodent chow and were allowed free access to water throughout the experiments. The rats were fed (Fed) or starved (Starved) for 24, 48, and 72 h. For analysis of tyrosine phosphorylation of hepatic proteins, at the indicated time, the rats were anesthetized with pentobarbital (50 mg of pentobarbital/kg of body weight), the abdominal cavity was opened, the portal vein was exposed, and 1 ml of PBS with or without 1.4 U of bovine insulin (Sigma Chemical Co., St. Louis, MO, U.S.A.) was injected. Each group consisted of 4 rats. After 30 seconds, the liver was quickly excised, put into liquid nitrogen, and stored at −80 °C until extraction. Liver extracts for immunoblotting were prepared as described previously.11 The protein concentration of the prepared liver extract was measured using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA, U.S.A.).

A solution of aPY (1:3000) or of an antibody against the carboxyl terminal amino acid sequence of IRS-1 (aIRS-1; 1 µg/ml, Seikagaku Co., Tokyo) diluted in the blocking buffer was used for immunodetection. The results were measured using the NIH Image computer program (Version 1.57). For the insulin binding assay, a semi-purified insulin receptor fraction from solubilized membranes from the livers of rats unfused insulin was prepared by the method of Havrankova et al.12 and Hedo et al.13 using wheat-germ agglutinin (WGA)-conjugated agaroose.12,13 Insulin was prepared as described by Furlanetto et al.14 and the insulin binding assay was done by the method of Balage et al.15

As preliminary experiments, we infused various concentrations of insulin into the portal vein and studied the course of changes in tyrosine phosphorylation of the insulin receptor and IRS-1 in intact liver. We observed that insulin injection increased tyrosine phosphorylation of these proteins in a manner responsive to dose, i.e., the ED50 was around 0.03 U, and 1.4 U of insulin was sufficient to give the maximum response within 30 s. Furthermore, tyrosine phosphorylation was maintained at the high level for at least 5 min (data not shown). Figure 1A shows that an injection of 1.4 U insulin into the portal vein stimulated tyrosine phosphorylation of the insulin receptor. In addition, starvation increased it significantly [Fed, 100.0 ± 9.9; Starved 24 h, 159.4 ± 8.9*; Starved 48 h, 158.8 ± 4.4*; Starved 72h, 171 ± 2.5*; values are means ± SEM for 4 rats, taking the mean value of the fed group as 100. Asterisks indicate a significant difference when compared with the value of

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Abbreviations: IRS-1, insulin receptor substrate-1; IRS-2, insulin receptor substrate-2; Shc, src homology/collagen; PBS, phosphate buffered saline; aIRS-1, anti-IRS-1 antibody; aIR, anti-insulin receptor antibody; aPY, anti-phosphotyrosine antibody; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; aIRS-1C, antibody against the carboxyl terminal amino acid sequence of IRS-1; WGA, wheat-germ agglutinin.
the fed group, \( p < 0.05 \) by Fisher’s PLSD (Stat View-J4.11, Abacus Concept, Inc., Berkeley, CA, U.S.A.). The data as described below were analyzed in the same way.] On the other hand, insulin binding of liver plasma membrane was measured by Scatchard plot analysis, and starvation did not affect the affinity of the receptor to insulin. However, the number of receptors was increased (Fed, 42.4 ± 1.8 fmol/mg protein; Starved 24 h, 67.0 ± 13.2; Starved 48 h, 112.4 ± 12.8\(^*\); Starved 72 h, 72.9 ± 4.1\(^*\)). When receptor phosphorylation was corrected for the amount of insulin binding activity, there was no change in the ratio of receptor phosphorylation to receptor in starved 24 h and 72 h groups, and a decrease in starved 48 h group (Fed, 1.00 ± 0.09; Starved 24 h, 1.01 ± 0.06; Starved 48 h, 0.60 ± 0.02\(^*\); Starved 72 h, 1.00 ± 0.03). These results suggested that the increase in overall autophosphorylation of the insulin receptor is primarily due to the increased number of receptors, as others have reported.\(^7\) In Fig. 1B, we measured the effect of starvation on insulin-dependent IRS-1 tyrosine phosphorylation. Starvation also increased the tyrosine phosphorylation of IRS-1 induced by insulin in the same manner as tyrosine phosphorylation of the insulin receptor (Fed, 100.0 ± 3.5; Starved 24 h, 121.1 ± 3.4\(^*\); Starved 48 h, 129.0 ± 4.5\(^*\); Starved 72 h, 144.6 ± 6.6\(^*\)). At the same time, we measured the amount of IRS-1 by immunoblotting (data not shown); this was not affected by starvation (Fed, 100.0 ± 9.3; Starved 24 h, 127.9 ± 21.2; Starved 48 h, 112.8 ± 18.6; Starved 72 h, 115.8 ± 18.0; these values are not significantly different). The ratio of tyrosine phosphorylation to the amount of IRS-1 was increased when starvation was prolonged (Fed, 1.00 ± 0.03; Starved 24 h, 0.95 ± 0.03; Starved 48 h, 1.14 ± 0.04\(^*\); Starved 72 h, 1.25 ± 0.06\(^*\)). These results are similar to the reports by Saad et al.\(^7\) Surprisingly, we found that the tyrosine phosphorylation of a 195-kDa protein was increased by starvation more prominently than that of IRS-1 (Fig. 2A, Fed, 100.0 ± 18.8; Starved 24 h, 267.4 ± 18.8\(^*\); Starved 48 h, 289.1 ± 27.0\(^*\); Starved 72 h, 348.7 ± 72.4\(^*\)). Because this protein is not recognized by the anti-IRS-1 antibody (Figs. 2A and 2B), we concluded that the 195-kDa protein is distinct from IRS-1. Our preliminary studies of the dose response of insulin and the course of insulin treatment showed that the tyrosine phosphorylation of this protein reflects the activation of insulin-receptor tyrosine kinase, suggesting that the 195-kDa protein is the direct substrate of the insulin-receptor tyrosine kinase. Based on our recent results,\(^10\) it is likely that the 195-kDa phosphotyrosyl protein stimulated by insulin is a new substrate of the insulin-receptor tyrosine kinase; however, we have not excluded the possibility that this protein is IRS-2.\(^9\) This is the first report showing that starvation strongly increases the insulin-dependent tyrosine phosphorylation of the 195-kDa protein.

Recently, we reported that treatment with cAMP-generating
agents potentiated the insulin-dependent tyrosine phosphorylation of the 195-kDa protein in primary cultures of rat hepatocytes, but not that of IRS-1 or IRS-2.\(^10\) Because starvation increased the cAMP concentration in liver (data not shown), the increase in hepatic cAMP concentration in response to starvation may affect the insulin-dependent tyrosine phosphorylation of the 195-kDa protein. At present, the distinct roles of IRS-1 and the 195-kDa protein remain to be discovered; however, the results showing that the changes in the insulin-dependent tyrosine phosphorylation of the 195-kDa protein are more marked in response to starvation than those of IRS-1, indicates that the tyrosine phosphorylation of the 195-kDa protein may be more important for insulin signal transmission under starvation conditions. Although the physiological meanings of these phenomena are unclear, it is possible that this increase is one of the mechanisms for attenuating the impairment of insulin action. Further studies will shed a light on the mechanisms of changes in insulin responsiveness in liver under various nutritional states such as starvation.

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