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DOI: 10.1590/S0100-879X1998001100007

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The insulin receptor substrate 1 associates with phosphotyrosine phosphatase SHPTP2 in liver and muscle of rats

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Abstract

Insulin stimulates the tyrosine kinase activity of its receptor resulting in the phosphorylation of its cytosolic substrate, insulin receptor substrate-1 (IRS-1) which, in turn, associates with proteins containing SH2 domains. It has been shown that IRS-1 associates with the tyrosine phosphatase SHPTP2 in cell cultures. While the effect of the IRS-1/SHPTP2 association on insulin signal transduction is not completely known, this association may dephosphorylate IRS-1 and may play a critical role in the mitogenic actions of insulin. However, there is no physiological demonstration of this pathway of insulin action in animal tissues. In the present study we investigated the ability of insulin to induce association between IRS-1 and SHPTP2 in liver and muscle of intact rats, by co-immunoprecipitation with anti-IRS-1 antibody and anti-SHPTP2 antibody. In both tissues there was an increase in IRS-1 association with SHPTP2 after insulin stimulation. This association occurred when IRS-1 had the highest level of tyrosine phosphorylation and the decrease in this association was more rapid than the decrease in IRS-1 phosphorylation levels. The data provide evidence against the participation of SHPTP2 in IRS-1 dephosphorylation in rat tissues, and suggest that the insulin signal transduction pathway in rat tissues is related mainly to the mitogenic effects of the hormone.

Key words

- Insulin action
- Insulin receptor substrate
- Phosphotyrosine phosphatase
- SHPTP2

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Presented at the XIII Annual Meeting
of the Federação de Sociedades de
Biologia Experimental, Caxambu,
MG, Brasil, August 26-29, 1998.

Research supported by FAPESP and
CNPq (PRONEX).

Received April 14, 1998
Accepted August 13, 1998

The insulin receptor is the principal mediator of insulin action on cellular and metabolic processes. The insulin receptor β -subunit, which contains an intrinsic tyrosine kinase, undergoes tyrosyl autophosphorylation and is activated in response to insulin binding to the extracellular α -subunit (1). Moreover the discovery of the tyrosine kinase activity of the insulin receptor sug-

gested that the mechanism of insulin action involved the tyrosyl phosphorylation of intracellular substrates. An insulin-stimulated phosphoprotein called pp185 was identified in many cells and tissues using anti-phosphotyrosine antibodies. One component of the pp185 band was purified and cloned from several sources (2-4) and the cloned protein was called insulin receptor substrate

1 (IRS-1). Evidence from different sources has shown that the phosphotyrosine-containing form of IRS-1 binds to the enzyme phosphatidylinositol 3-kinase (PI 3-kinase) through the Src homology 2 (SH2) domains of the latter (4,5) and that this association activates the enzyme.

In addition to PI 3-kinase, other proteins containing SH2 domains such as SHPTP2, Nck, Grb2 have been shown to bind to IRS-1 (1). SHPTP2 is an SH2 domain-containing tyrosine phosphatase that associates with the COOH-terminal tyrosine phosphorylation sites of IRS-1 in cell cultures (6), and this association increases the phosphatase activity of SHPTP2 *in vitro* (7). However, there is no physiological demonstration of this pathway of insulin action in animal tissues. In the present study we investigated the ability of insulin to induce association of IRS-1 and SHPTP2 in liver and muscle of intact rats, two of the main target tissues for insulin action.

The rats were anesthetized with sodium amobarbital (15 mg/kg body weight, intraperitoneally) and used in the experiments 10-15 min later, as soon as anesthesia was assured by the loss of foot and corneal reflexes. The abdominal cavity was opened, the vena cava exposed and 0.5 ml saline (0.9% NaCl), containing or not 6 µg of insulin, was injected. After the indicated time, the liver or muscle was removed, minced coarsely and immediately homogenized in extraction buffer (1% Triton X-100, 100 mM Tris, pH 7.4, containing 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM PMSF and 0.1 mg aprotinin/ml) at 4°C with a Polytron PTA 20S generator (Brinkmann Instruments model PT 10/35; Westbury, NY) operated at maximum speed for 30 s. Both extracts were centrifuged at 15,000 rpm and 4°C in a Beckman 70.1 Ti rotor (Palo Alto, CA) for 45 min to remove insoluble material, and the supernatant of these tissues was used for immunoprecipita-

tion with anti-IRS-1 antibody and protein A Sepharose 6 MB. The samples were treated with Laemmli sample buffer (8) containing 100 mM DTT, heated in a boiling water bath for 4 min and submitted to SDS-PAGE (6.5% Tris/acrylamide). Electrotransfer of proteins from the gel to nitrocellulose was performed for 2 h at 100 V (constant) in the Bio-Rad miniature transfer apparatus (Mini-protean), as described by Towbin et al. (9) but with 0.02% SDS added to the transfer buffer to enhance the elution of high-molecular mass protein. Nonspecific protein binding to nitrocellulose was reduced by pre-incubating the filter overnight at 4°C in blocking buffer (3% BSA, 10 mM Tris, 150 mM NaCl and 0.02% Tween 20). The pre-stained molecular mass standards used were myosin (205 kDa), galactosidase (116 kDa), BSA (80 kDa) and ovalbumin (49.5 kDa). The nitrocellulose blot was incubated with anti-phosphotyrosine (1 µ/ml) or anti-SHPTP2 (1:100) antibodies for 4 h at 22°C. Monoclonal anti-phosphotyrosine antibody and anti-IRS-1 and anti-SHPTP2 antibodies were from Santa Cruz Biotechnology, Santa Cruz, CA. The blots were subsequently incubated with 2 µCi [¹²⁵I]-protein A (30 µCi/µg) in 10 ml of blocking buffer for 1 h at 22°C and washed again. [¹²⁵I]-protein A bound to the antibodies was detected by autoradiography using preflashed Kodak XAR film with Cronex Lightning Plus intensifying screens at -70°C for 12-48 h. Band intensities were quantified by optical densitometry (Molecular Dynamics) of the developed autoradiogram (10-12).

To estimate the rate of insulin-induced IRS-1 phosphorylation in the liver, we performed a time course experiment after the administration of insulin into the portal vein. Liver extracts were immunoprecipitated with anti-IRS-1 antibody and blotted with anti-phosphotyrosine antibody. As shown in Figure 1A, 30 s after exposure to insulin there was a substantial increase in the phosphorylation of IRS-1, the rate of which decreased

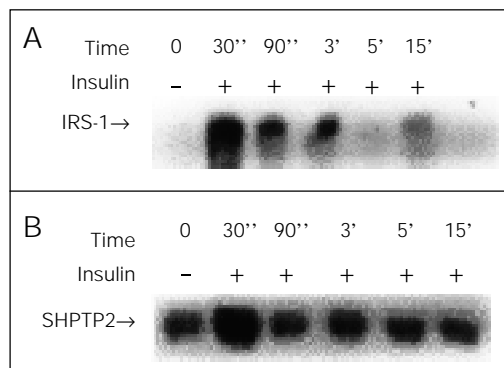


Figure 1 - Time course of insulin-stimulated IRS-1 tyrosine phosphorylation (panel A) and association with SHPTP2 in rat liver (panel B). Rats were anesthetized and the abdominal wall was incised to expose the viscera. Saline or insulin was infused at the indicated time. The tissues were excised and homogenized in extraction buffer at 4°C as described in the text. After centrifugation, aliquots containing equal amounts of protein were immunoprecipitated with anti-IRS-1 antibody and protein A Sepharose 6 MB and then resolved on 6.5% SDS-polyacrylamide gels. The protein bands were subsequently transferred to a nitrocellulose membrane and detected with anti-phosphotyrosine antibody (A) or anti SHPTP2 (B) and [¹²⁵I]-protein A, after which the membrane was subjected to autoradiography. The data are representative of five experiments.

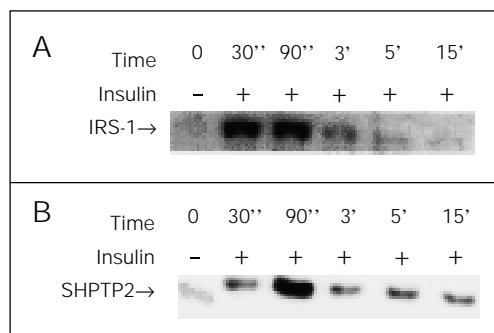


Figure 2 - Time course of insulin-stimulated IRS-1 tyrosine phosphorylation (panel A) and association with SHPTP2 in rat muscle (panel B). Rats were anesthetized and the abdominal wall was incised to expose the viscera. Saline or insulin was infused at the indicated time. The tissues were excised and homogenized in extraction buffer at 4°C as described in the text. After centrifugation, aliquots containing equal amounts of protein were immunoprecipitated with anti-IRS-1 antibody and immunoblotted as described in the legend to Figure 1. The data are representative of four experiments.

thereafter and had almost vanished by 15 min. In order to investigate the association of SHPTP2 with IRS-1 the same membrane was stripped and re-blotted with anti-SHPTP2 antibody. Figure 1B shows that there is a basal association between IRS-1 and SHPTP2 which increases 30 s after insulin infusion and returns to basal levels thereafter. It is interesting to note that at 90 s and 3 min after insulin infusion there still was IRS-1 tyrosine phosphorylation, but the association with SHPTP2 was just basal.

The results obtained for muscle were similar to those obtained for liver but high tyrosine phosphorylation levels of IRS-1 were observed at 30 and 90 s after insulin infusion (Figure 2A). When these samples previously immunoprecipitated with anti-IRS-1 antibody were blotted with anti-SHPTP2 antibody, an important association was observed 30 s

after insulin infusion, which was dissociated from IRS-1 tyrosine phosphorylation, whose levels continued to be high up to 90 s.

The results presented here show that SHPTP2 associates with phosphorylated IRS-1 in liver and muscle of rats. In cell culture, SHPTP2 is activated during association with IRS-1. While the effect of IRS-1/SHPTP2 association on signal transmission is not completely known, there is a possibility that this interaction autoregulates IRS-1 phosphorylation. The tyrosine phosphatase SHPTP2 may dephosphorylate signaling intermediates located either in the IRS-1 signaling complex or at distant sites, thus downregulating signaling. In this regard, there is a study showing that SHPTP2 is able to dephosphorylate IRS-1 *in vitro* (13), but this phenomenon was not observed *in vivo* (14-16). Our results showing that IRS-1/SHPTP2

association occurs when IRS-1 has the highest level of tyrosine phosphorylation are evidence against the effect of SHPTP2 dephosphorylating IRS-1, and suggest that this association depends on an important increase in IRS-1 tyrosine phosphorylation.

The role of SHPTP2 in insulin signaling has been examined by several approaches. Microinjection of the SH2 domains of SHPTP2 or antibodies against SHPTP2 blocks insulin-stimulated DNA synthesis (14,17). Similarly, overexpression of a catalytically inactive mutant SHPTP2 molecule inhibits mitogenesis and p21^{ras} and MAP kinase activation (14-17). It is not clear how SHPTP2 transmits signals to p21^{ras} and MAP kinase or mediates mitogenesis during insulin signaling, but there is no doubt that the activity of SHPTP2 plays a critical role in the mitogenic actions of insulin.

It is known that insulin activates hexose transport via at least two mechanisms: a p21^{ras}-dependent pathway leading to an increase in the amount of cell surface GLUT 1,

and a metabolic, p21^{ras}-independent pathway leading to translocation of the insulin-responsive transporter GLUT 4 to the cell surface (1,7). Hausdorff et al. (17), using microinjection of a glutathione S-transferase fusion protein encoding the N- and C-terminal SH2 domains of SHPTP2 or anti-SHPTP2 antibodies into 3T3-L1 adipocytes, demonstrated that SHPTP2 is important for the expression of GLUT 1, but is not required for activation of GLUT 4 translocation. These data suggest that SHPTP2 plays a role in the insulin-induced transcription of immediate early genes such as GLUT 1, but is not required for the metabolic increase in transport mediated by GLUT 4 translocation.

In summary, the present results demonstrate that after insulin stimulation of liver and muscle of intact rats there is an increase in the association of IRS-1 with SHPTP2, a pathway that has been shown to play an important role in insulin-induced DNA synthesis in cell culture.

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