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ORNITHINE DECARBOXYLASE INDUCTION DURING LIVER REGENERATION IN IRS-1-DEFICIENT MICE

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SUMMARY: We investigated the induction of ornithine decarboxylase during liver regeneration after partial hepatectomy in IRS-1-deficient mice. There were no significant differences in ODC activity or the time course of changes in ODC activity between IRS-1-deficient mice and wild-type mice. PI 3'-kinase activity showed similar increases in both groups of mice. Furthermore, ODC induction in IRS-1 transfected CHO cells was studied after stimulation by addition of FCS. The maximal ODC activity was 2.5-fold greater in IRS-1-transfected CHO cells than in control CHO cells. Our results suggest that the IRS-1 pathway may be involved in ODC induction. The absence of a difference in ODC and PI 3'-kinase activity in the regenerating liver between IRS-1-deficient mice and wild-type mice may have been related to the compensatory effects of IRS-2/pp190 [Araki et al. Nature (1994) 372, 186-190; Tobe et al. J.Biol.Chem. (1995) 270, 5698-5701].

Insulin induces a wide variety of growth and metabolic responses in a number of types of cells. Insulin's biological effects are initiated by activation of tyrosine kinase in the β-subunit and

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Abbreviations : IRS-1, insulin receptor substrate-1; ODC, ornithine decarboxylase; PI 3'-kinase, phosphatidylinositol 3'- kinase; FCS, fetal calf serum.

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phosphorylation of a number of proteins including IRS-1. IRS-1 binds several SH2 proteins via tyrosine phosphorylation sites and is considered to be the core of the signaling complex (1). Insulin induces ODC, a key enzyme of polyamine metabolism, and associates to cell proliferation (2).

We previously observed marked enhancement of tyrosyl-phosphorylation of IRS-1 during liver regeneration, suggesting that IRS-1 may be involved in transmission of the insulin signal to intracellular regulators involved in hepatocyte growth (3). Growth hormone, which promotes early expression of the hepatocyte growth factor gene expression (4), has also been reported to induce tyrosyl-phosphorylation of IRS-1(5). These findings suggest that induction of tyrosine phosphorylation of IRS-1 by insulin, growth hormone, and other unidentified hormonal and growth factors may be an important event in hepatocyte growth. We investigated induction of ODC, which is induced by insulin stimulation and has been observed during liver regeneration after partial hepatectomy (6), in IRS-1-deficient mice to clarify the role of IRS-1 in hepatocyte proliferation.

EXPERIMENTAL PROCEDURES

In vivo experiments Partial hepatectomies were performed in wild-type mice weighing 20g and mice with the targeted disruption of the IRS-1 gene (7). Liver tissue was homogenized in 25mM Tris HCl (pH 7.2) containing 0.2 M sucrose and 50mM KCl and then centrifuged at100,000 g. The resulting supernatant was used in assays of ODC and protein. For assay of PI 3'-kinase, liver tissue was homogenized in 25mM Tris HCl (pH 7.5) containing 10mM sodium orthovanadate, 10mM sodium pyrophosphate, 100mM sodium fluoride, 10mM EDTA, 10mM EGTA and 1mM PMSF and centrifuged in an Eppendorf-type microcentrifuge. The supernatants were incubated with anti-phosphotyrosine antibodies (PY20 or PT66, Transduction Laboratories, KY, USA or BioMakor, Israel, respectively). After the addition of Persorbin, (Boehringer Mannheim) the PI 3'-kinase activity in the immunoprecipitates was determined according to a previously described method (8).

In vitro induction of ODC CHO cells overexpressing human insulin receptors (CHO/IR) and both IR and IRS-1 (CHO/IR.IRS-1) were incubated overnight in FCS-free Ham F12 and then stimulated with Ham F12 containing 10% FCS. ODC activity was measured according to a previously described method (9). One unit of ODC activity was defined as the amount releasing 1 nmol CO_2/hour at 37°C. Immunoprecipitation with anti IRS-1 antibody, #1-6 (10) and Western blot analysis using the anti-IRS-1 monoclonal antibodies 6B7 (11) or PT66 were performed according to previously described methods (10).

RESULTS

ODC induction during liver regeneration after partial hepatectomy in wild-type and IRS-1 deficient mice

The maximum ODC activity occurred 6 hours after surgery in both IRS-1-deficient and wild-type mice (Fig.1). There was no significant difference in the time course of changes in ODC activity between groups.

Activation of PI 3'-kinase after partial hepatectomy in wild type and IRS-1 deficient mice

PI 3'-kinase was similarly activated 1 hour after hepatectomy in both groups of mice with the use of two different kinds of anti phosphotyrosine monoclonal antibodies, PT 66 and PY 20 (Fig.2).

ODC induction in CHO/IR and CHO/IR.IRS-1 cells

ODC was induced by the addition of 10 % FCS to the culture medium of CHO/IR and CHO/IR.IRS-1 cells. Tyrosine-phosphorylation of IRS-1 was induced by both 10 % FCS stimulation and insulin stimulation, although insulin induced a greater degree of tyrosine phosphorylation (Fig.3). Both CHO/IR

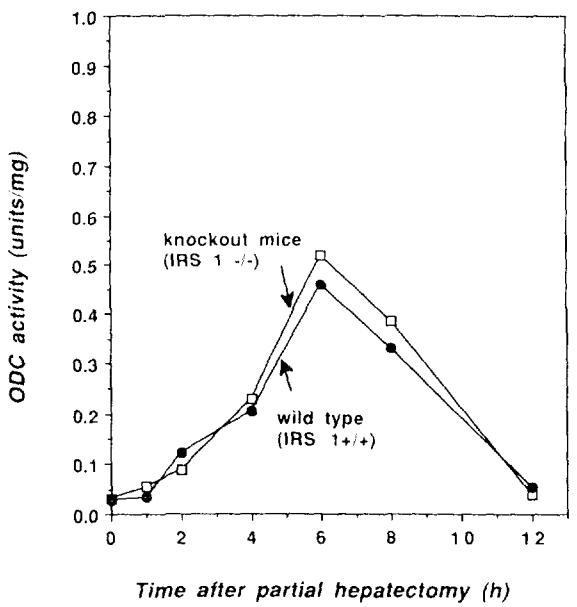

Figure 1. ODC induction in IRS-1-deficient (knockout mice) and wild type mice during liver regeneration after partial hepatectomy. Values are the mean of values in 3 mice at 0,1,2,8 and 12 h and 5 mice at 4 and 6 h.

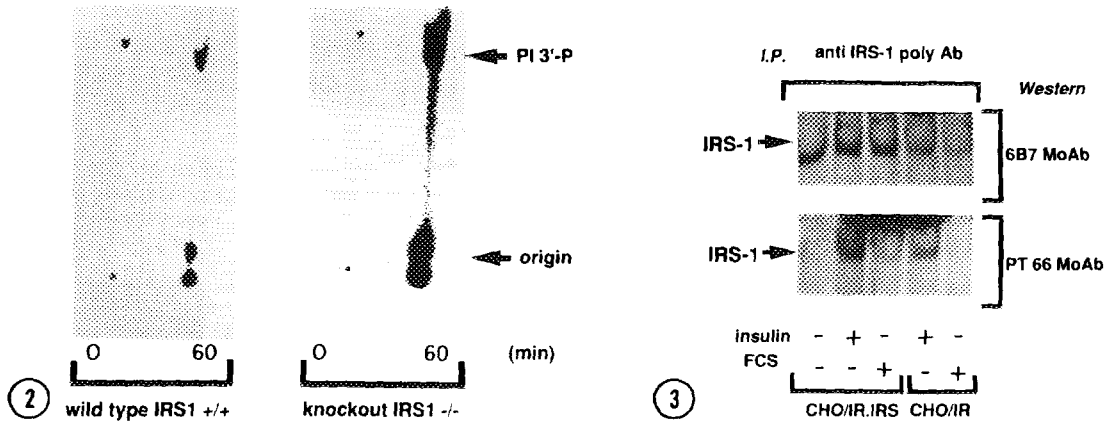

Figure 2. Representative assay of PI 3'-kinase activity in IRS-1-deficient (knockout mice) and wild-type mice after partial hepatectomy. PI 3'-kinase activity was measured at 0 and 60 minutes after partial hepatectomy using the anti-phosphotyrosine monoclonal antibody PT66.

Figure 3. The effect of insulin and FCS on tyrosine-phosphorylation of IRS-1in insulin receptor-expressing CHO cells (CHO/IR) and insulin receptor- and IRS-1-expressing CHO cells (CHO/IR.IRS-1). Cells were incubated in 268 nM insulin or 10% FCS for 10 min at 37°C.

and CHO/IR.IRS-1 cells showed maximal ODC activity 4 hours after the addition of FCS with the specific activitis of 3.5 and 5.0 units/mg, respectively (Fig. 4-A).

In preliminary experiment, we found that CHO/IR.IRS-1 cells were larger than CHO/IR cells (data not shown). When the maximum specific activity (units/mg) was converted to the maximal activity per number of cells (units/ 1 X 10^6 cells), specific activity at the time of maximal ODC activity in CHO/IR.IRS-1 cells was 2.5-fold greater than in control CHO cells (3.28 ± 0.96 units/1 X 10^6 cells vs. 1.46 ± 0.19units/1 X 10^6 cells, $p<0.05$) (Fig. 4-B).

DISCUSSION

We previously observed a marked enhancement of tyrosyl-phosphorylation of IRS-1 during regeneration of the liver, which suggests that IRS-1 may play an important role in transmitting the insulin signal to the intracellular regulators involved in hepatocyte growth (3). In the present study, we attempted to elucidate the functional role of IRS-1 in the regenerating liver by measuring the changes in ODC activity that are induced during liver regeneration (6). ODC activity was similar in both IRS-1-deficient and wild-type

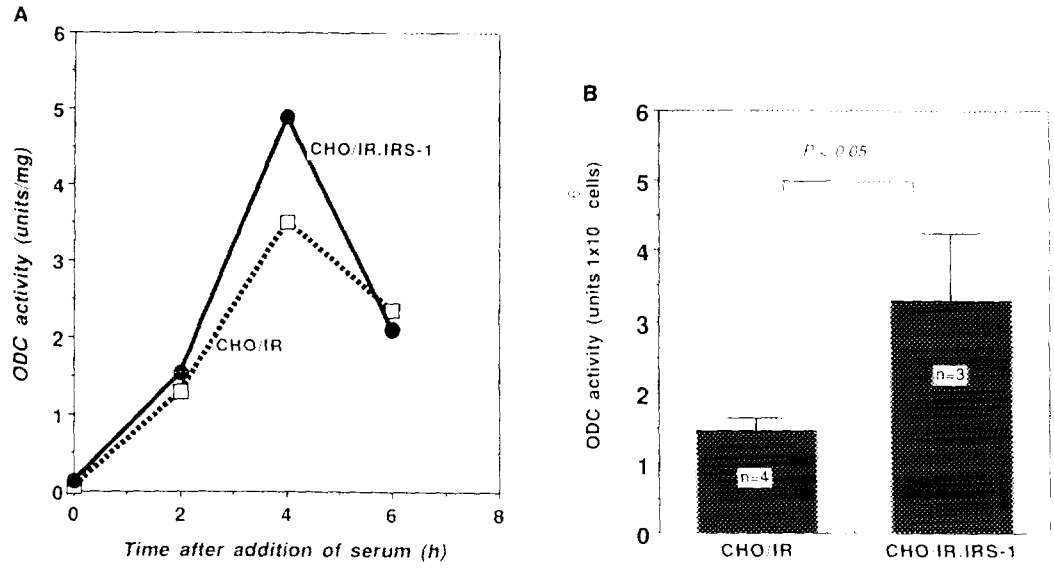

Figure 4.-A ODC induction after the addition of 10% FCS in insulin receptor-expressing CHO cells (CHO/IR) and insulin receptor-and IRS-1-expressing CHO cells (CHO/IR.IRS-1). Values are the mean of duplicate experiments.
-B ODC activity per number of cells 4 hours after the addition of FCS.

mice, suggesting that IRS-1 is not involved in ODC induction in the regenerating liver. However, two recent studies have suggested that an alternative pathway IRS-2/pp190, is enhanced in IRS-1-deficient mice and that IRS-2/pp190 may substitute for IRS-1 function (12,13). PI 3'-kinase associating with tyrosyl-phosphorylated IRS-1 is activated during regeneration of the liver following partial hepatectomy (3). Thus, we suggest that the activation of PI 3'-kinase in IRS-1-deficient mice may have been induced by tyrosyl-phosphorylated IRS-2/pp190. Tobe et al. reported that insulin stimulation had the same effect on IRS-2/pp190 and IRS-1 in respect of the association with Grb-2/Ash and PI 3'-kinase (13).

Insulin causes a marked induction of ODC (2), but the precise mechanisms of its action are unknown. Insulin preferentially stimulates the translation of ODC mRNA through phosphorylation of eIF4B and 4E in murine fibloblast cells expressing the human insulin receptor (14), and ODC induction is stimulated in cells overexpressing eIF-4E (15). Recent studies have shown that 4E-BP1, which is a substrate for MAP kinase, directly associats eIF 4E (16,17). MAP-kinase and 4E-BP1 appear to be located downstream

of IRS-1. MAP-kinase activity has been found to be regulated by tyrosine-phosphorylated IRS-1-Grb2-SOS complex (18). These observations suggest that the IRS-1 pathway may be at least partially responsible for the induction of ODC.

In the present study, tyrosine-phosphorylation of IRS-1 in CHO/IR.IRS-1 and CHO/IR cells was lower with FCS stimulation than with insulin stimulation. This finding may have been related to the lower concentration of insulin in the culture medium. However, ODC induction was very weak in the presence of insulin alone (data not shown). In contrast, Manzella et al found that insulin induced ODC activity in NIH 3T3 cells expressing the human insulin receptor (14). The discrepancy in these results may be due to differences in cell types. It is possible that insulin alone which plays as a progression factor is insufficient to induce ODC in the G_1 phase of the cell cycle (6) and that an additional gowth factor, which acts as a competent factor in FCS, is required.

Our results indicate that the IRS-1 pathway is involved in ODC induction. The absence of a difference in ODC activity in the regenerating liver between IRS-1-deficient mice and wild-type mice may have been related to the compensatory effects of IRS-2/pp190. However it is possible that the mechanisms of ODC induction in the regenerating liver after partial hepatectomy and in the insulin stimulation may not be the same.

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