

# Tissue-specific regulation of IRS-1 in unilaterally nephrectomized rats

A.D. Sasse, E. Chen,  
C.R.O. Carvalho,  
J.A.R. Gontijo, S.L. Brenelli  
and M.J.A. Saad

Departamento de Clínica Médica, Faculdade de Ciências Médicas,  
Universidade Estadual de Campinas, Campinas, SP, Brasil

## Abstract

Insulin stimulates the tyrosine kinase activity of its receptor, resulting in the phosphorylation of its cytosolic substrate, insulin receptor substrate 1 (IRS-1). IRS-1 is also a substrate for different peptides and growth factors, and a transgenic mouse “knockout” for this protein does not have normal growth. However, the role of IRS-1 in kidney hypertrophy and/or hyperplasia was not investigated. In the present study we investigated IRS-1 protein and tyrosine phosphorylation levels in the remnant kidney after unilateral nephrectomy (UNX) in 6-week-old male Wistar rats. After insulin stimulation the levels of insulin receptor and IRS-1 tyrosine phosphorylation were reduced to  $79 \pm 5\%$  ( $P < 0.005$ ) and  $58 \pm 6\%$  ( $P < 0.0001$ ), respectively, of the control (C) levels, in the remnant kidney. It is possible that a circulating factor and/or a local (paracrine) factor playing a role in kidney growth can influence the early steps of insulin action in parallel. To investigate the hypothesis of a circulating factor, we studied the early steps of insulin action in liver and muscle of unilateral nephrectomized rats. There was no change in pp185 tyrosine phosphorylation levels in liver (C  $100 \pm 12\%$  vs UNX  $89 \pm 9\%$ , NS) and muscle (C  $100 \pm 22\%$  vs UNX  $91 \pm 17\%$ , NS), and also there was no change in IRS-1 phosphorylation levels in both tissues. These data demonstrate that after unilateral nephrectomy there is a decrease in insulin-induced insulin receptor and IRS-1 tyrosine phosphorylation levels in kidney but not in liver and muscle. It will be of interest to investigate which factors, probably paracrine ones, regulate these early steps of insulin action in the contralateral kidney of unilaterally nephrectomized rats.

## Key words

- Insulin receptor
- Insulin receptor substrate
- Unilateral nephrectomy
- Insulin action

## Correspondence

M.J.A. Saad  
Departamento de Clínica Médica  
Faculdade de Ciências Médicas  
Universidade Estadual de Campinas  
13083-970 Campinas, SP  
Brasil  
Fax: 55 (019) 239-3114

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One of the earliest cellular responses to stimulation by insulin is the activation of insulin receptor kinase and tyrosine phosphorylation of insulin receptor  $\beta$  subunit and pp185, a cytoplasmic phosphoprotein found in most cells and tissues (1). A component of the pp185 band was purified and cloned from several sources (2,3). The cloned pro-

tein was called insulin receptor substrate 1 (IRS-1). This insulin receptor substrate is a cytoplasmic protein that is rapidly phosphorylated at a specific tyrosine after insulin stimulation and is important in mediating the metabolic and growth-promoting effects of this hormone. IRS-1 is also a substrate for different peptides and growth factors (1,2,4,5)

including IGF-1 and GH, and a transgenic mouse "knockout" for this protein does not have normal growth. IGF-1 and its receptor have been involved in kidney compensatory hypertrophy after unilateral nephrectomy (UNX) or diabetes. However, the role of IRS-1 in kidney hypertrophy and/or hyperplasia was not investigated. The first aim of this study was to investigate IRS-1 protein and tyrosine phosphorylation levels in the remnant kidney after UNX.

Male Wistar rats (6 weeks old) underwent unilateral left nephrectomy (the kidneys were trimmed of fat and capsule and the adrenal glands were left intact), as described previously (6), or sham operation, where a flank incision was made and the left kidney was manipulated, but not removed. The animals recovered and received food and water *ad libitum*. After seven days, the rats were anesthetized with sodium amobarbital (15 mg/kg body weight, intraperitoneally) and used in experiments 10-15 min later, as soon as anesthesia was confirmed by the loss of pedal and corneal reflexes. The abdominal cavity was opened, the vena cava exposed and 0.5 ml of normal saline (0.9% NaCl) containing or not 10  $\mu$ M insulin was injected. After 30 or 90 s, fragments of liver and muscle, respectively, were removed, minced coarsely and immediately homogenized in approximately 6 volumes of solubilization buffer A using a Polytron PTA 20S generator (Brinkmann Instruments, model PT 10/35) operated at maximum speed for 30 s in a water bath maintained at 100°C as previously described (7-9). To investigate IRS-1 phosphorylation in kidney, saline with or without insulin was infused into the vena cava and the remnant kidney was extracted 90 s later in the same way as described for liver and muscle. Solubilization buffer A contained 1% SDS, 50 mM HEPES, pH 7.4, 100 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, and 10 mM sodium vanadate. The homogenates were

then boiled for 10 min and cooled in an ice bath for 40 min. In some experiments, kidney, liver and muscle were excised and homogenized with a Polytron apparatus in 6 volumes of homogenization buffer B cooled in an ice bath. The composition of buffer B was the same as buffer A except that 1% Triton X-100 replaced 1% SDS and 2 mM PMSF and 0.1 mg/ml aprotinin were added. Both extracts were centrifuged at 100,000 g (55,000 rpm) at 4°C in a Beckman 70.1 Ti rotor for 30 min to remove insoluble material, and the resulting supernatant was used for the experiments. The kidney, liver and muscle homogenized in buffer B were used for immunoprecipitation with anti-IRS-1 antibody and Protein A-Sepharose 6 MB.

The samples were treated with Laemmli sample buffer (10) containing 100 mM DTT and heated in a boiling water bath for 4 min. For total extracts, similar size samples (150  $\mu$ g of protein) were submitted to SDS/PAGE (6.5% Tris/acrylamide) in a Bio-Rad miniature slab gel apparatus (11). 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. (12) but with 0.02% SDS added to the transfer buffer to enhance the elution of high molecular mass protein. Nonspecific protein binding to the nitrocellulose was reduced by preincubating the filter overnight at 4°C in blocking buffer (3% BSA, 10 mM Tris, 150 mM NaCl, and 0.02% Tween 20). The prestained molecular mass standards used were myosin (205 kDa),  $\beta$ -galactosidase (116 kDa), BSA (80 kDa) and ovalbumin (49.5 kDa). The nitrocellulose blot was incubated with anti-phosphotyrosine antibody (1  $\mu$ g/ml) for 4 h at 22°C. The blots were subsequently incubated with 2  $\mu$ Ci of  $^{125}$ I-Protein A (30  $\mu$ Ci/ $\mu$ g) in 10 ml of blocking buffer for 1 h at 22°C and washed for 2 h.  $^{125}$ I-Protein A bound to the antibodies was detected by autoradiography using preflashed Kodak

XAR film with Cronex Lightning Plus intensifying screens at  $-70^{\circ}\text{C}$  for 12-48 h. Band intensities were quantified by densitometry (Molecular Dynamics) of the developed autoradiogram.

In the present study we evaluated the effect of UNX on insulin receptor and IRS-1 phosphorylation in the remnant kidney. There was no change in insulin receptor protein levels as determined by immunoblotting with an antibody to the C-terminus of the insulin receptor (data not shown). Following insulin infusion into the vena cava, a phosphotyrosine band of 95 kDa, previously identified as the insulin receptor  $\beta$  subunit (1,13,14), appeared and became prominently phosphorylated. The level of phosphorylation of this band was reduced to  $79 \pm 5\%$  ( $P < 0.005$ ) in the remnant kidney. In addition to the 95-kDa band seen after insulin injection, a broad band migrating between 165 and 185 kDa was also detectable (Figure 1A). This band is known as pp185 and IRS-1 is one component of this band (15). The phosphorylation of pp185 was reduced to  $58 \pm 6\%$  ( $P < 0.0001$ ) in the remnant kidney. In order to characterize IRS-1 phosphorylation after insulin stimulation, we immunoprecipitated kidney extracts with anti-IRS-1 antibody and immunoblotted these with anti-IRS-1 and anti-phosphotyrosine antibody (Figure 1B). There was no change in IRS-1 protein levels in the remnant kidney. However, after insulin stimulation IRS-1 tyrosine phosphorylation was reduced to  $41 \pm 5\%$  ( $P < 0.0001$ ) in the kidney of UNX rats compared to the kidney of control rats. The mechanism(s) responsible for the reduction in insulin receptor and IRS-1 tyrosine phosphorylation in the remnant kidney after 7 days of UNX have not been elucidated. The regulation of insulin receptor and IRS-1 is under the control of different situations such as fasting, obesity, hormones and diabetes. We have previously demonstrated that in other tissues the excess of cortisol (16), glucagon (7), epinephrine

(9), and GH (17) and also hyperinsulinemia (13) can modulate the early steps of insulin action by inducing a reduction in insulin receptor and IRS-1 phosphorylation. However, none of these hormones is in excess in our animal model of kidney growth. It is possible that a circulating factor and/or a local (paracrine) factor that plays a role in kidney growth can influence the early steps of insulin action in parallel. If there is a

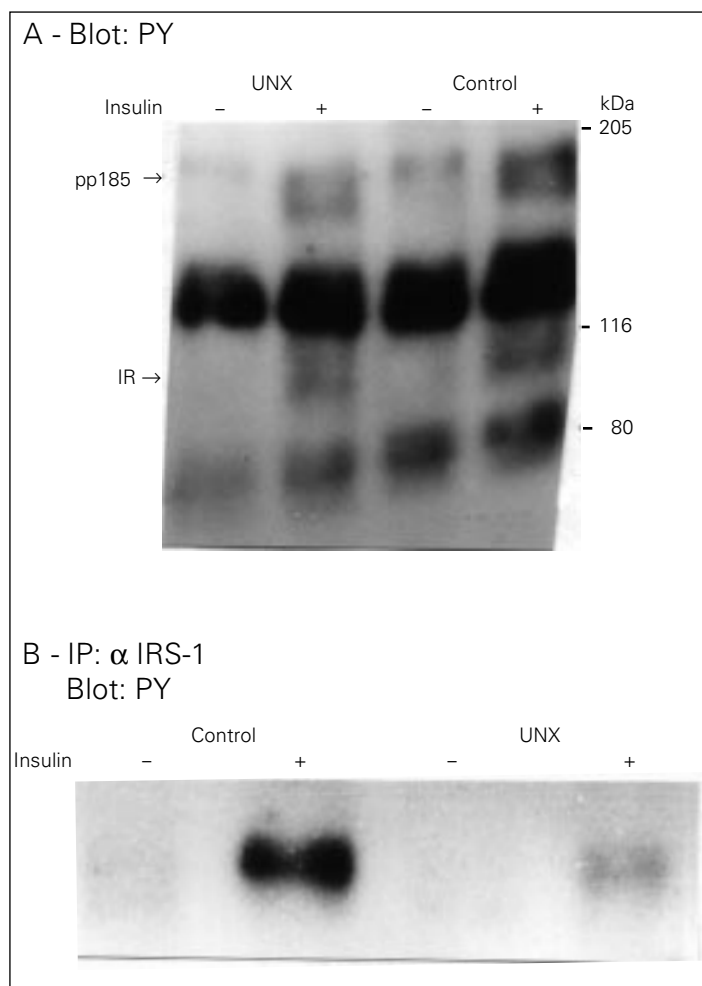
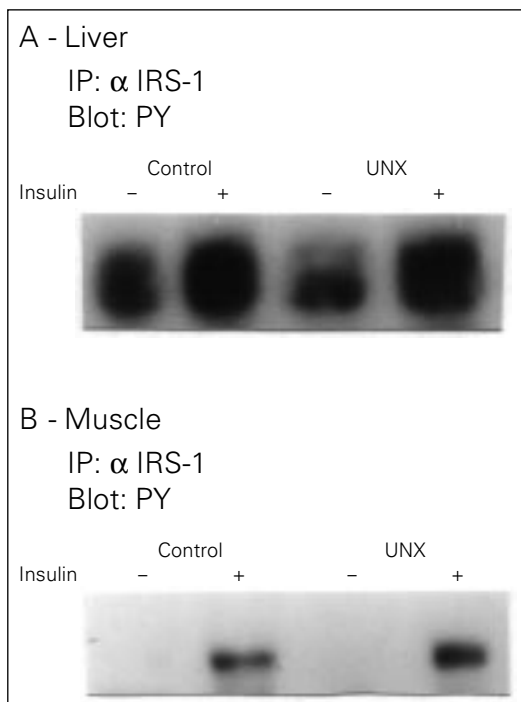


Figure 1 - A, Insulin receptor (IR) and pp185 tyrosine phosphorylation in remnant kidney tissue after saline (-) or saline plus 10  $\mu\text{M}$  insulin (+) in unilaterally nephrectomized (UNX) rats, compared with controls. The tissue was extracted as described in Methods and immunoblotting (Blot) of total tissue extracts was performed with anti-phosphotyrosine antibody (PY) and  $^{125}\text{I}$ -Protein A. B, Effect of unilateral nephrectomy on insulin receptor substrate 1 (IRS-1) in the rat remnant kidney. Kidney extracts from controls and unilaterally nephrectomized rats were immunoprecipitated (IP) with anti-IRS-1 antibody ( $\alpha$  IRS-1) and then immunoblotted with anti-phosphotyrosine antibody.

Figure 2 - Effect of unilateral nephrectomy (UNX) on insulin receptor substrate 1 (IRS-1) in the rat liver and muscle. The liver extracts from controls and unilaterally nephrectomized rats were immunoprecipitated (IP) with anti-IRS-1 antibody ( $\alpha$  IRS-1) and then immunoblotted (Blot) with anti-phosphotyrosine antibody (PY) (A). The same procedure was applied to muscle (B).



circulating factor that can regulate the early steps of insulin action in kidney, it may also modulate insulin receptor and IRS-1 in other tissues such as liver and muscle. To investigate this possibility we studied the early steps in insulin action in liver and muscle of

unilateral nephrectomized rats. The experiments were performed as described above, except that we infused insulin into the portal vein, and extracted liver and muscle as described above. The results of total tissue extracts showed that in liver there was no change in pp185 phosphorylation levels ( $C 100 \pm 12\%$  vs  $UNX 89 \pm 9\%$ , NS). In accordance, after immunoprecipitation with anti-IRS-1 antibody and blotting with anti-phosphotyrosine antibody there was also no change in IRS-1 tyrosine phosphorylation in the liver of UNX rats (Figure 2A). In muscle the results were very similar with no change in insulin-induced pp185 phosphorylation ( $C 100 \pm 22\%$  vs  $UNX 91 \pm 17\%$ , NS) and also in IRS-1 tyrosine phosphorylation (Figure 2B) in UNX rats.

In summary, the data demonstrated that after UNX there was a decrease in insulin-induced insulin receptor and IRS-1 tyrosine phosphorylation levels in the remnant kidney but not in liver and muscle. It will be of interest to determine which factors, probably paracrine ones, regulate these early steps of insulin action in the contralateral kidney of UNX rats.

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