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Activation of phosphatidylinositol-3-kinase by insulin is mediated by both A and B human insulin receptor types

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Activation of a phosphatidylinositol-3-kinase (PI-3-kinase) is one of the earliest consequences of insulin binding to the receptor. The human insulin receptor exists in two isoforms which differ in the length of the α-subunit (HIR-A = 719 aa, HIR-B = 731 aa). To test whether both isoforms transduce an insulin signal on PI-3-kinase we used rat-1-fibroblasts expressing HIR-A or HIR-B. We found that insulin stimulates ^{32}P incorporation into PIP through both HIR-A and HIR-B to a similar extent (approx. 8-10 fold).

The human insulin receptor exists in two forms which differ in the length of the α-subunit (HIR-A = 719 aa, HIR-B = 731 aa) (1,2). Both receptor types are expressed in different human tissues to a different proportion. There is also some evidence for different functional properties of both receptor types (3,4). It is, however, not known at present whether the two receptor types transduce the same spectrum of signals or couple to different "postkinase signal transducers". One potentially important signal transducing system in insulin action recently described is a phosphatidylinositol (PtdIns) 3-kinase that phosphorylates the D-3 position of the inositol ring (5,6). This kinase seems to be associated with growth related proteins (7,8) containing tyrosine kinase activity, and its stimulation by insulin in cultured cells represents one of the strongest biological effects of the hormone. Using rat 1 fibroblasts transfected with HIR-A or HIR-B

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respectively we investigated whether both types of insulin receptors are able to stimulate the PtdIns-3-kinase activity.

Materials and Methods

Materials: Porcine insulin was purchased from Novo Industrie (Denmark). (gamma-^{32}P)ATP was obtained from NEN. Phenylmethane sulphonyl fluoride, nonidet P-40, L-a-phosphatidylinositol and phosphoinositides were from Sigma. Protein A-agarose was purchased from Oncogene Science, Dianora GmbH. Aprotinin was from Bayer (Germany). Silica gel 60F_{254} thin layer plates came from Merck (Darmstadt).

Cell culture: Rat 1 fibroblasts transfected with HIR-A and B respectively were cultured in D-MEM medium F12 (Gibco), containing 10 % (vol/vol) fetal bovine serum. Cells were grown to confluence in 4 mm dishes and then incubated overnight in MEM medium containing 0.5 % albumin (quiescing medium).

PtdIns-3-kinase assay: After incubation with insulin, the cells were washed once with phosphate buffered saline, and lysed at 4°C for 20 min in 1 ml of the buffer described in (6). Lysates were centrifuged at 10 000 g for 10 min, the supernatants were incubated for 1 hr with anti-phosphotyrosine antibodies (70μg/ml of lysate) prepared as in (9), and then for 1,5 hr with Protein-A agarose. Immunoprecipitates were washed as in (6) and pellets were directly incubated with PtdIns (0.1 mg/ml) for 20 min in medium containing 25 mM Hepes, pH 7.4, and 1.2 mM sodium orthovanadate. Phosphorylation was started by addition of 50 μM (gamma-^{32}P)ATP and 5 mM $MgCl_2$ and the reaction was carried out at 22°C for 20 min in a final volume of 50 μl. After addition of 150 μl of 1N HCl, lipides were extracted with 300 μl of Chloroform:Methanol (1:1 by vol), the organic phase was washed thrice with 150 μl of Methanol:1N HCL (1:1 by vol), and phospholipids were separated by TLC as in (9). Lipids were detected with iodine vapor and the ^{32}P-labelled spots by autoradiography. The spots corresponding to labelled PtdIns-P (PIP) were scraped out and counted by liquid scintillation. Phosphoinositides were used as standards in each chromatogram.

Results and Discussion

The existence of two types of HIR differing in the C-terminal part of the α-subunit, the fact that they are differentially expressed in different tissues and the differences in their functional properties raise the question whether this domain restricts the ability of the receptor isotypes to initate only certain specific intracellular effects. The use of cell lines tranfected with only one of these receptor types should allow to undertake studies to resolve this issue. In the present work we used rat 1 fibroblasts transfected with HIR-A or HIR-B respectively and we investigated the stimulation of PtdIns-3-kinase by insulin in both cases. This enzymatic activity has

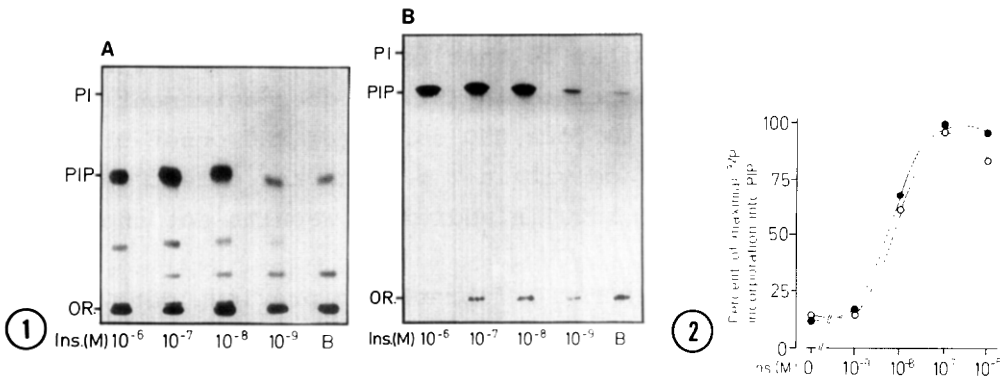

Fig. 1A and B
Autoradiograms showing the effect of insulin on ^{32}P incorporation into phosphatidylinositol-phosphate. Rat-1 fibroblasts expressing HIR-A (A) or HIR-B (B) were stimulated with insulin in the indicated concentrations for 10 minutes. Insulin receptor and PI-kinase were then isolated and PI-kinase activity was measured as described in the Methods section. Each experiment was confirmed twice.

Fig. 2
Dose response curves of the insulin effect on ^{32}P incorporation into phosphatidyl-inositolphosphate in Rat-1 fibroblasts expressing HIR-A (o - o) or HIR-B (● - ●).

been recently reported to be highly increased by insulin in transfected CHO cells (5,6), representing one of the strongest cellular effects of the hormone so far known. Rat 1 fibroblasts were incubated at 37°C for 10 min in the presence of different insulin concentrations, and pellets obtained after immunoprecipitation with anti-P-Tyr antibodies were used as source of the lipid kinase as indicated in the Methods section. Fig. 1A and B show an autoradiography of phosphorylated lipids comigrating with PtdIns P. As can be observed ^{32}P incorporation into PtdIns occurs in both cases in an insulin-dependent manner confirming previously reported data for CHO cells. Radioactive PtdIns-P formed from PtdIns was quantitated by liquid scintillation and the results are shown in Fig. 2, expressed as percent of maximal ^{32}P incorporation for each type of cells. Both curves are similar indicating that coupling between insulin binding and lipid kinase stimulation is mediated by both types of receptor with a roughly similar sensitivity to the hormone. The maximal effect is reached at 10^{-7}M insulin with concentrations of the hormone of 4nM giving approximately a half-maximal effect on

PtdIns-3-kinase activity. Insulin caused a 8-10 fold stimulation in both cases, similar to that reported in (6) but lower than the stimulation described in (5). In good agreement with previously published data form CHO-cells (6) the onset of the insulin effect takes place within 2 min and the effect decreases at 30 min after insulin addition (results not shown) in both cell types.

These results confirm in rat 1 fibroblasts previous data obtained with CHO cells and support the idea that PtdIns-3-kinase is a cellular target of insulin receptor kinase that is activated either by tyrosine phosphorylation or by tight association with the phosphorylated form of the ß-subunit. To our knowledge this is the first study that compares in parallel the ability of both insulin receptor types in mediating a biological effect of the hormone. Our data suggest that the absence or presence of the 12 C-terminal amoniacids in the α-subunit has no consequences for coupling the insulin binding to PtdIns-3-kinase stimulation.

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