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Differential Effects of IGF-I, IGF-II and Insulin in Human Preadipocytes and Adipocytes – Role of Insulin and IGF-I Receptors

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Abstract

We compared insulin and IGF effects in adipocytes expressing IR (insulin receptors), and preadipocytes expressing IR and IGF-IR (IGF-I receptors).

Treatment of adipocytes with insulin, IGF-II or IGF-I resulted in phosphorylation of IR. Order of potency was insulin>IGF-II>IGF-I. In preadipocytes IR, IGF-IR and insulin/IGF-I hybrid receptors (HR) were detected. Treatment of preadipocytes with IGF-I and IGF-II 10^{-8} M resulted in activation of IGF-IR and IR whereas insulin was more potent in activating IR, with no effect on IGF-IR.

In adipocytes glucose transport was 100-fold more sensitive to insulin than to IGFs and the maximal effect was higher with insulin. In preadipocytes glucose accumulation and DNA synthesis was equally sensitive to insulin and IGFs but the maximal effect was higher with IGF-I. In conclusion, insulin and IGF-I activate their cognate receptors and IGF-I also HR. IGF-II activates IR, IGF-IR and HR. Insulin and IGF-I are partial agonists to each other's receptors.

1. Introduction

Insulin, insulin-like growth factor-I (IGF-I) and insulin-like growth factor-II (IGF-II) are all part of the insulin-like growth factor family and involved in growth, metabolism and survival of the cell (Navarro et al., 1999; Saltiel, Kahn, 2001; Siddle et al., 2001; Chao, D'Amore, 2008). IGF-II is the predominant IGF in humans with circulating levels 5-10-fold higher than IGF-I (Frystyk, 2004; Hedman et al., 2004). In contrast to circulating IGF-I, which peaks during puberty, IGF-II is constantly expressed throughout life (Juul et al., 1995).

The IR and the IGF-IR are both members of the tyrosine kinase family of receptors and share an overall homology of more than 50% (Ullrich et al., 1986). As a result of dissimilarities in structure there are differences in tyrosine phosphorylation of the receptors (Lammers et al., 1989). IGF-IR primarily mediates mitogenic effects, while IR primarily mediates metabolic effects (Blakesley et al., 1996; Kim, Accili, 2002). Differences in signalling between the two receptors can also be due to differences in distribution of the IR and IGF-IR in different cell types, receptor activation, rate of internalization and intracellular signal transduction (Blakesley et al., 1996; Kim, Accili, 2002; Werner et al., 2008). The $\alpha\beta$ -heterodimers from IR and IGF-IR respectively can combine to form insulin/IGF-I hybrid receptors (Moxham et al., 1989). Insulin, IGF-I and IGF-II all bind to IGF-IR and IR but with different affinities, as reviewed by Werner et al (Werner et al., 2008). The IR has a high affinity for insulin, but can also bind IGF-II with 10-50 fold lower affinity and IGF-I with 100-500 fold lower affinity. IGF-II binds to the IGF-IR with 10-fold lower affinity compared with IGF-I. Insulin/IGF-I hybrid receptors function mainly as IGF-IR with much higher affinity for IGF-I and IGF-II than for insulin (Soos et al., 1993; Slaaby et al., 2006). This would imply that the effect of the ligand varies with different receptor distributions.

Our aim was to compare the effects of insulin, IGF and IGF-II and study the impact of differences in distribution of IGF-IR and IR on their action in adipocytes, which express almost exclusively IR, and human preadipocytes, which express both of IR and IGF-IR (Bäck, Arnqvist, 2009).

2. Materials and methods

2.1. Cell culture

Cell culture flasks and plates were purchased from Corning (Schiphol-Rijk, The Netherlands) and all other cell culture materials were purchased from Invitrogen (Paisley, UK) or as indicated in the text. Human preadipocytes and adipocytes were isolated from subcutaneous adipose tissue from elective abdominal surgery. None of the patients had known diabetes. The average BMI was 26.7 kg/m^2 , range $21.1\text{--}31.2 \text{ kg/m}^2$, except for one patient who was extremely obese, BMI 49.1 kg/m^2 . All patients had given their informed consent, the procedures were approved by the local ethics committee at Linköping University and were performed in accordance with the Declaration of Helsinki.

2.2. Isolation of human adipocytes and preadipocytes

Fat pads were isolated and cut with scissors before incubation with 300 u/ml collagenase type 1 (Sigma-Aldrich Sweden AB, Stockholm, Sweden) in a Krebs-Ringer solution (0.12 M NaCl, 4.7 mM KCl, 1.2 mM KH_2PO_4 , 1.2 mM MgSO_4 and 2.5 mM CaCl_2) with addition of 20 mM HEPES, pH 7.40, (Sigma), 2 mM glucose, 200 nM adenosine and 1% (w/v) fatty acid-free bovine serum albumin (BSA). Digested tissue was filtered through gauze to remove connective tissue and cells were washed three times with the Krebs-Ringer solution. Fat cells

were then isolated by flotation, a procedure which has been shown to give pure adipocytes (Rodbell, 1964). Isolated adipocytes were incubated in 37°C over night to reverse insulin resistance induced by surgical stress, as previously described in (Danielsson et al., 2005). Preadipocytes were isolated from the Krebs-Ringer solution after the adipocytes were removed by floatation essentially as described in (Halvorsen et al., 2001). Briefly, the cell suspension was centrifuged at 500xg for 5 min and the pellet washed once with preadipocyte culture medium (equal amounts of DMEM and Ham's F-12, 10% (v/v) newborn calf serum (NCS), 100 iu/ml penicillin, 100 µg/ml streptomycin, 2 µg/ml Fungizone®) and then cultured in 75 cm² culture flasks with preadipocyte culture medium. Medium was changed 2-3 times a week and cells were passaged using trypsin-EDTA when nearly confluence. Cells were not used for more than 5 passages.

2.3. Cell incubation

Preadipocytes were serum-deprived over night in DMEM containing 100 iu/ml penicillin, 100 µg/ml streptomycin, 2 µg/ml Fungizone® and 0.1% (w/v) BSA. Insulin, IGF-I or IGF-II at indicated concentrations were added to the flasks and they were incubated at 37°C for 10 min. The medium was removed and lysis buffer (pH 7.5) containing 20 mM TrizmaBase, 150 mM NaCl, 5 mM EDTA, 0.5% (w/v) sodiumdeoxycholate and 0.5% (v/v) Triton X-100 with addition of 1 mM phenylmethylsulphonyl fluoride (PMSF), 1.5 µg/ml leupeptin, 1 mM Na₃VO₄ and 1.5 µg/ml aprotinin was added to the flasks. The cells were lysed on ice for 30 min and then harvested by scraping. The lysate was centrifuged at 12000xg for 15 min at 4°C and the supernatant was stored at -70°C until use.

The adipocytes were preincubated for 15 min with 100 nM phenylisopropyl adenosine and 0.5 u/ml adenosine deaminase and then with indicated concentrations of insulin, IGF-I or IGF-II for a further 10 min. The media were removed and the cells resuspended in the lysis buffer.

The cells were homogenized using a potter-elvehjem grinder and the lysate was stored frozen until analyzed.

2.4. Immunoprecipitation, SDS-PAGE and immunoblotting

Lysates were immunoprecipitated (IP) with either rabbit polyclonal anti-IR β antibody (c-19) or rabbit polyclonal anti-IGF-IR β antibody (c-20) (Santa Cruz Biotechnology, Heidelberg, Germany), diluted 1:400, and incubated at 4°C for 2 h. 20 mg/ml Protein A Sepharose (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) in 0.1% (w/v) BSA in the lysis buffer was then added and the lysates were incubated overnight at 4°C with gentle rocking. The next day lysates were centrifuged at 6500xg for 5 min, supernatants were removed and pellets were washed three times with the lysis buffer. Pellets were then dissolved in SDS-PAGE sample buffer (6.25 mM TrizmaBase, 1% (w/v) SDS, 10% (v/v) glycerol, 0.001% (v/v) bromophenol) with addition of 2% (v/v) β -mercaptoethanol and boiled for 3 minutes. Immunoprecipitated proteins were separated in 7.5% acrylamide gels and proteins electrophoretically blotted to polyvinylidene difluoride (PVDF) membranes. After blocking in 3% (w/v) bovine serum albumin, membranes were incubated with antiphosphotyrosine (PY20) or, after stripping membranes in 62 mM Tris-HCl, 2% (w/v) sodium dodecylsulfate, 100 mM β -mercaptoethanol for 30 minutes at 56°C, with anti-IGF-IR or anti-IR antibodies. Bound antibodies were detected using horseradish peroxidase-conjugated secondary antibodies and the ECL-plus kit of GE Healthcare. For densitometric evaluation autoradiographs were scanned with a LAS1000 camera (Fujifilm, Tokyo, Japan) and then analyzed using the Multi Gauge software, version 3.0 (Fujifilm). Protein phosphorylation was normalized to total receptor protein and data was expressed as % of maximum effect or as % of unstimulated control.

2.5. Determination of thymidine incorporation in preadipocytes

Cultures of preadipocytes were grown in 6-well plates until near confluence. The cells were incubated with insulin and IGF-I at indicated concentrations in serum free DMEM overnight and then with 1 $\mu\text{Ci/ml}$ [$6\text{-}^3\text{H}$]-thymidine for 3 hours at 37°C , 5% CO_2 . Cells were washed and DNA was then precipitated in ice cold 5% (w/v) trichloroacetic acid for 15 min at 4°C . After treatment with 0.1 M potassium hydroxide for 1-2 hours at room temperature 800 μl of the lysate was added to 4 ml of of UltimaGoldTM scintillation fluid (Chemical Instruments AB (CiAB), Sollentuna, Sweden) and the radioactivity was measured. Data were expressed as percent of radioactivity in untreated control cells.

2.6. Determination of glucose accumulation in preadipocytes

Glucose accumulation was determined as described previously (Bäck, Arnqvist, 2009).

Briefly, the cells were grown in 6-well plates, serum-deprived overnight and then incubated for 3 h in serum free DMEM (glucose concentration 1000 mg/l) with addition of 0.2 $\mu\text{Ci/ml}$ D-[U- ^{14}C]-glucose and insulin or IGF-I at indicated concentrations. The cells were lysed in 0.1% sodium dodecylsulfate and the radioactivity was measured. Data were expressed as percent of radioactivity in unstimulated control cells. EC_{50} values, maximum values and confidence intervals were calculated with GraphPad Prism 4.00 (GraphPad Software Inc., San Diego, CA, USA).

2.7. Determination of glucose transport in adipocytes

Glucose uptake was determined as uptake of 2-deoxy-D-[1- ^3H]glucose (Amersham Biotech, Uppsala, Sweden) after transfer of cells to medium without glucose (Karlsson et al., 2004). Adipocytes at 10% (v/v) were incubated at 37°C with the indicated concentration of insulin, IGF-I or IGF-II for 15 min, when 2-deoxy-D-[1- ^3H]glucose was added to a final

concentration of 50 μM (10 $\mu\text{Ci/mL}$) and the cells were incubated for a further 30 min. Incubations were terminated by separating the cells from the medium during 5-second centrifugation through dinonylphtalate oil. The cell cake was dissolved in sodium dodecyl sulfate (SDS) and the amount of radioactivity in the cells determined by scintillation counting. Radioactivity in the medium trapped between cells was corrected for by subtracting radioactivity in incubations that were immediately terminated by addition of cytochalasin B and spinning through the oil. EC_{50} values, maximum values and confidence intervals were calculated with GraphPad Prism 4.00 (GraphPad Software Inc., San Diego, CA, USA).

2.8. Statistical analysis

Statistical comparisons were made with SPSS 17.0 (SPSS Inc. Headquarters, Chicago, Illinois, USA) by ANOVA. For post hoc analysis Dunnet's test was used. When indicated a nonparametric test, related-samples Wilcoxon signed ranks test, was used. GraphPad Prism 4.00 (GraphPad Software Inc., San Diego, CA, USA) was used for comparison of dose response curves when indicated, and for drawing bar graphs. A p value <0.05 was considered statistically significant. Data is presented as mean $\pm$ SE.

3. Results

3.1. Preadipocytes

3.1.1. Receptor phosphorylation

In preadipocytes we detected IGF-IR after immunoprecipitation with IR antibody and then immunoblotting with an IGF-IR antibody, and vice versa, indicating the presence of insulin/IGF-I hybrid receptors.

Preadipocytes were stimulated with either IGF-II, IGF-I or insulin at 10^{-9} - 10^{-8} M (Figure 1), then the receptor proteins were immunoprecipitated either with an antibody against the IGF-IR β -subunit or with an antibody against the IR β -subunit and analyzed by SDS-PAGE and immunoblotting. After immunoprecipitation with the antibody against IGF-IR β we could detect phosphorylation by IGF-II 10^{-8} M, IGF-I 10^{-8} M, inconsistently also by IGF-I 10^{-9} M, but not by insulin. Immunoprecipitation with the antibody against IR β revealed phosphorylation by IGF-II 10^{-8} M, insulin 10^{-9} - 10^{-8} M and IGF-I 10^{-8} M.

3.1.2. Thymidine incorporation

To quantify DNA synthesis in preadipocytes the incorporation of [6- 3 H]-thymidine was examined after the cells were stimulated with insulin, IGF-I or IGF-II at 10^{-11} - 10^{-7} M (Figure 2A). IGF-I 10^{-8} M, IGF-II 10^{-8} M or IGF-I 10^{-7} M significantly stimulated DNA synthesis. The effect of IGF-I tended to be higher than that of insulin.

To further examine the higher maximal effects of IGF-I compared to insulin a new set of experiments were performed to see if the effect of IGF-I was additional to the effect of insulin. Cells were treated with insulin 10^{-8} - 10^{-5} M alone or with IGF-I 10^{-8} or 10^{-7} M added (Figure 2B). As seen in Figure 2A a maximal effect of IGF-I alone was obtained at a concentration of 10^{-8} M and did not increase further at 10^{-7} M. With insulin alone a maximal effect was obtained at 10^{-6} M and then levelled off.

The addition of IGF-I at the concentration of either 10^{-8} or 10^{-7} both resulted in an additional effect compared to insulin alone (Figure 2B). To compare the maximal effects the peak of each curve was compared to the other's in each experiment. Using paired t-test a p-value of

0.059 was found and when using related-samples Wilcoxon signed ranks test, a nonparametric test, a significant difference ($p=0.046$) was found between the highest effect of insulin alone (10^{-6} M) and the highest effect of insulin + IGF-I 10^{-8} M obtained at insulin 10^{-7} M. At insulin concentrations above 10^{-7} M the effect of adding IGF-I, either 10^{-8} M or 10^{-7} M, started to decrease.

3.1.3. Glucose accumulation

The accumulation of D-[U- 14 C]-glucose was investigated to examine the metabolic effects of IGF-II, insulin and IGF-I in preadipocytes (Figure 3). The cells were stimulated with IGF-II, insulin or IGF-I at 10^{-11} - 10^{-7} M. IGF-II 10^{-8} - 10^{-7} M, insulin 10^{-8} - 10^{-7} M or IGF-I 10^{-9} - 10^{-7} M significantly stimulated glucose accumulation in comparison with unstimulated controls. The EC_{50} values were similar for all three conditions, $1.29 \times 10^{-9} \pm 0.24 \times 10^{-9}$ M for IGF-II, $0.89 \times 10^{-9} \pm 0.03 \times 10^{-9}$ M for insulin, and $0.73 \times 10^{-9} \pm 0.03 \times 10^{-9}$ M for IGF-I. However, the maximal response obtained by IGF-I was higher than the responses obtained by IGF-II or insulin. The max effects and 95% confidence interval for the max effects (expressed as percent above control) were for IGF-II 79.4 (63.9-94.8), for IGF-I 104.9 (85.1-124.8) and for insulin 59.3 (46.0-72.6).

3.2. Adipocytes

3.2.1. Receptor phosphorylation

Adipocytes were stimulated with insulin at 10^{-9} - 10^{-8} M, or IGF-I or IGF-II at 10^{-9} - 10^{-7} M (Figure 4). Receptor proteins were then immunoprecipitated and analyzed with SDS-PAGE and immunoblotting.

IGF-IR protein was barely demonstrable and phosphorylation of the IGF-IR could not be detected. The IR was phosphorylated in response to IGF-I at 10^{-7} M, IGF-II at 10^{-8} - 10^{-7} M, insulin at 10^{-9} - 10^{-8} M, and inconsistently by IGF-I at 10^{-8} M.

3.2.2. Glucose transport

We examined glucose uptake in response to insulin, IGF-I or IGF-II in order to investigate the metabolic effects of these stimuli on human adipocytes (Figure 5). IGF-II had an EC_{50} value of 3×10^{-9} M, the EC_{50} value of insulin was 20×10^{-12} M and for IGF-I it was 6×10^{-9} M. The max effects and 95% confidence interval for the max effects (expressed as percent above control) were for IGF-II 95.8 (18.7-172.9), IGF-I 87.0 (74.1-99.9) and for insulin 139.9 (103.8-176.0).

4. Discussion

We recently showed that human adipocytes contain very few IGF-IRs in comparison to IRs, whereas human preadipocytes exhibit both receptors (Bäck, Arnqvist, 2009). This gives an opportunity to study the impact of differences in receptor distribution on the effects of insulin and IGFs. In this study adipocyte IGF-IR protein was barely demonstrable and phosphorylation of IGF-IR was not detected. In preadipocytes IGF-II at 10^{-8} M activated both the IR and IGF-IR. To our knowledge effects of IGF-II on human preadipocytes have not been reported before. It should be noted that the IR exists in two isoforms due to alternative splicing of the IR gene. Isoform B contains the 12 amino acids encoded by the exon that has been spliced off in isoform A. IGF-II has a higher affinity for IR-A than for IR-B (Belfiore et al., 2009). In adipose tissue both IR-A and IR-B are present (Sesti et al., 1995). We have

recently shown that isolated adipocytes express mostly IR-A and few IR-B, while preadipocytes express only IR-A (Bäck, Arnqvist, 2009).

No effect of insulin at 10^{-9} - 10^{-8} M was found on the phosphorylation of IGF-IR in preadipocytes, but insulin is known to phosphorylate the IGF-IR at higher concentrations (Chisalita et al., 2006). The IR in preadipocytes was phosphorylated in response to insulin at physiological concentrations (10^{-9} - 10^{-8} M). IGF-I at 10^{-8} M activated the IR in preadipocytes but not in adipocytes. After immunoprecipitation with antibodies specific for the IGF-IR β -subunit we could detect both the IGF-IR β -subunit and the IR β -subunit on the gel and after immunoprecipitation with antibodies specific for the IR β -subunit we could detect both the IR β -subunit and the IGF-IR β -subunit. In mature adipocytes only antibodies specific for the IR β -subunit precipitated the IR β -subunit and no IGF-IR β -subunit was detected on the gel. This shows that some of the receptors on preadipocytes, but not on adipocytes, are insulin/IGF-I hybrid receptors. Hybrid receptors have been reported to function as IGF-IR thus having a high affinity for IGF-I but not for insulin (Soos et al., 1993). The fact that the IR β -subunit immunoprecipitated with antibodies against the IGF-IR was not activated by insulin (Figure 1) and that the IR was activated by IGF-I 10^{-8} M in preadipocytes but not in adipocytes is probably explained by insulin/IGF-I hybrid receptors (Frattali, Pessin, 1993; Engberding et al., 2009).

Glucose uptake in preadipocytes tended to increase at 10^{-9} M insulin and was significantly stimulated at 10^{-8} - 10^{-7} M with an EC_{50} value of 0.9×10^{-9} M. As preadipocytes mainly express glucose transporter-1 (GLUT1) and not the insulin-regulated GLUT4 (Hauner et al., 1998; Bäck, Arnqvist, 2009), these effects of insulin in the preadipocytes could be due to relocation of GLUT1 in combination with enhanced metabolism of glucose. IGF-I or IGF-II stimulated glucose uptake with similar EC_{50} values as insulin, 0.7×10^{-9} M and 1.3×10^{-9} M, respectively.

IGF-I, IGF-II and insulin also stimulated DNA synthesis in a similar way. With regard to the metabolic and mitogenic effects preadipocytes exhibit similar sensitivity for IGF-I, IGF-II and insulin. Although comparable effects of insulin, IGF-I and IGF-II were found in mitogenic and metabolic assays the initiation of the effects were probably different. Judging from the receptor phosphorylation assays, insulin activated IR, IGF-I activated IGF-IR and insulin/IGF-I hybrid receptors while IGF-II activated IR, IGF-IR and insulin/IGF-I hybrid receptors. Our experiments do not allow us to conclude which insulin receptor isoform that is involved in the IGF-II effects in preadipocytes.

In adipocytes the stimulation of glucose transport was about 100-fold more sensitive to the action of insulin than to the effects of IGF-I and IGF-II. EC_{50} values for adipocytes glucose accumulation, observed by Sinha et al (Sinha et al., 1989; Sinha et al., 1990), were 3×10^{-11} - 6×10^{-11} M for insulin, 2×10^{-9} M for IGF-II and 2×10^{-8} M for IGF-I. This is well in agreement with our results (2×10^{-11} M for insulin, 3×10^{-9} M for IGF-II and 0.6×10^{-8} M for IGF-I). IGF-II is known to have a higher affinity for IR than IGF-I (Denley et al., 2004) and in our study IR was phosphorylated at a 10-fold lower concentration of IGF-II than of IGF-I. Our study shows that when preadipocytes are developed into mature adipocytes there is a huge increase in their response to insulin. This suggests that the recruitment of preadipocytes into adipocytes is a critical step in the regulation of adiposity.

In preadipocytes the maximal effects of IGF-I on glucose accumulation and DNA synthesis were higher than the maximal effect of insulin. When studied on thymidine incorporation in preadipocytes IGF-I in combination with insulin gave an additional effect compared to the maximal effect of insulin alone. This suggests that insulin is only a partial agonist to the IGF-IR and insulin/IGF-I hybrid receptor, and that IGF-I has other properties than insulin when

interacting with its cognate receptor, which could possibly be of importance in malignancies (Frasca et al., 2008). An augmented mitogenic effect of IGF-I compared to insulin has also been observed in vascular smooth muscle cells (Bornfeldt et al., 1991) and in cultured human skeletal muscle IGF-I has an increased mitogenic activity compared with insulin (Ciaraldi et al., 2001). It is believed that the C-domain of IGF-I somehow is important for interaction with the IGF-I receptor as suggested by Denley (Denley et al., 2004) and that this may in part explain the differences in maximal effects between IGF-I and insulin. Another very potent activator of mitogenic signalling via the IGF-IR is IGF-II. However IGF-II does not interact with the CR-domain of the IGF-IR and it is therefore likely that other structural determinants of the IGF-IR are of importance for mitogenic signalling (Ward, Lawrence, 2009). There are also other differences in the interaction of IGF-I with the IGF-IR or IR which affect binding affinity and influence signalling (De Meyts, Whittaker, 2002; Ward, Lawrence, 2009). In human adipocytes the maximal effect of insulin on glucose transport was significantly higher than the effects of IGF-I. There are few comparisons of maximal effects of insulin and IGFs in adipocytes in the literature and the results are not conclusive (DiGirolamo et al., 1986; Bolinder et al., 1987; Sinha et al., 1989).

When thymidine incorporation was studied with high concentrations of ligand the effect levelled off. This was seen with insulin alone and with IGF-I added to insulin. This may be explained by negative cooperativity (De Meyts, Whittaker, 2002). It is also possible that high concentrations of stimuli may induce negative feedback signals (Danielsson et al., 2006).

In conclusion our results suggest that insulin and IGF-I activate their cognate receptors and IGF-I also insulin/IGF-I hybrid receptors whereas IGF-II at physiological concentrations can activate both insulin and IGF-I receptors as well as insulin/IGF-I hybrid receptors. Insulin and IGF-I are only partial agonists to each other's receptors.

Declaration of interest

The authors declare that there is no conflict of interest.

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Figure legends

Figure 1

Phosphorylation of IGF-IR and IR in human preadipocytes. Cell lysate was immunoprecipitated (IP) with polyclonal antibodies against either the IGF-I receptor (IGF-IR) β -subunit (c-20) (A-B) or the insulin receptor (IR) β -subunit (c-19) (C-D). Immunoblotting with a phosphotyrosine antibody (PY-20) was used to analyze the phosphorylation. Results are mean $\pm$ SE from 3-6 separate experiments with preadipocytes from different subjects. * p <0.05 in comparison with unstimulated control. ** p <0.001 in comparison with unstimulated control.

Figure 2

(A) Effects of IGF-II (gray bars), insulin (white bars) and IGF-I (black bars) on the incorporation of [6-³H]-thymidine in human preadipocytes. Near confluent cells were stimulated with peptides in serum-free DMEM over night and then incubated with [6-³H]-thymidine for 3 h. Results are mean $\pm$ SE from 6 separate experiments with preadipocytes from different subjects. * p <0.05 in comparison with unstimulated control. ** p <0.001 in comparison with unstimulated control.

(B) Effects of insulin (white bars), insulin + IGF-I 10^{-8} (gray bars) or insulin + IGF-I 10^{-7} (black bars) on the incorporation of [6-³H]-thymidine in human preadipocytes. Near confluent cells were stimulated with peptides in serum-free DMEM over night and then incubated with [6-³H]-thymidine for 3 h. Results are mean $\pm$ SE from 6 separate experiments with preadipocytes from different subjects.

Figure 3

Effects of IGF-II (■), IGF-I (▲) and insulin (●) on the accumulation of D-[U-¹⁴C]-glucose in human preadipocytes. Near confluent cells were incubated at 37°C for 3 h with D-[U-¹⁴C]-glucose and polypeptides.

Results are mean $\pm$ SE from 6 separate experiments with preadipocytes from different subjects. * p <0.05 in comparison with unstimulated control. ** p <0.001 in comparison with unstimulated control.

Figure 4

Phosphorylation of IR in human adipocytes. Cell lysate was immunoprecipitated (IP) with polyclonal antibodies against the insulin receptor (IR) β -subunit (c-19). Immunoblotting with a phosphotyrosine antibody (PY-20) was used to analyze the phosphorylation. Cells were stimulated with insulin, IGF-I or IGF-II at 10^{-9} , 10^{-8} (A) or 10^{-7} (C) M. Gels were analysed densitometrically (B). Results are mean $\pm$ SE from 3 separate experiments. Adipocytes isolated from the same individual were used for control and stimulation by the ligand (IGF-I, IGF-II or insulin). To get enough material adipocytes from all in all 10 different patients had to be used. * p <0.05 in comparison with unstimulated control.

Figure 5

Primary human adipocytes was incubated with the indicated concentrations of IGF-II (■), IGF-I (▲) or insulin (●) for 15 min and the glucose transport was determined as the uptake of 2-deoxy-D-[1-³H]glucose. Results are expressed as % above unstimulated control, mean of 5 (insulin) or 4 (IGF-I and IGF-II) subjects $\pm$ SE. * p <0.05 in comparison with unstimulated control. ** p <0.001 in comparison with unstimulated control.

Highlights

In preadipocytes IR, IGF-IR and insulin/IGF-I hybrid receptors were detected.
Insulin and IGF-I activate their cognate receptors and IGF-I also hybrid receptors.
IGF-II activates IR, IGF-IR and insulin/IGF-I hybrid receptors.
Insulin and IGF-I are partial agonists to each other's receptors.

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Figure 3

Figure 5