Diabetologia (1996) 39: 1432–1439

Diabetologia

Comparison of the effects of insulin, PDGF, interleukin-6, and interferon-γ on glucose transport in 3T3-L1 cells: lack of cross-talk between tyrosine kinase receptors and JAK/STAT pathways

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Summary The effects of insulin, insulin-like growth factor (IGF)-I, platelet-derived growth factor (PDGF), interleukin (IL)-6 and interferon-γ on 2-deoxyglucose uptake and insulin receptor substrate (IRS)-1 phosphorylation were compared in 3T3-L1 cells at confluence and after differentiation to the adipocyte-like phenotype. Insulin and IGF-I produced the expected stimulation of glucose transport and tyrosine phosphorylation of IRS-1 in both confluent and differentiated cells. In contrast, IL-6 and interferon-γ failed to stimulate glucose transport or IRS-1 phosphorylation, although a marked stimulation of the JAK/STAT pathways as shown by acute-phase response factor (APRF)/Stat3 or Stat1 activation was observed in fibroblasts (IL-6, interferon-γ) and adipocytes (IL-6). PDGF-AA and PDGF-BB stimulated glucose transport in confluent, undifferentiated cells to the same extent as insulin (approximately six-fold stimulation), but produced only a small portion of the effect of insulin in differentiated cells. Similarly, mRNA levels and autophosphorylation of PDGF receptors were much lower in differentiated cells than in confluent fibroblasts. In contrast to insulin and IGF-I, PDGF failed to stimulate tyrosine phosphorylation of IRS-1. All effects of insulin, IGF-I, and PDGF on glucose transport were inhibited by Wortmannin; the half-maximally inhibiting concentration (IC_{50}) of Wortmannin was increased by insulin. These data demonstrate distinct signalling potentials of the investigated receptors, and indicate that the IL-6 and interferon-γ controlled JAK/STAT pathways lack the potential to stimulate glucose transport. IRS-1 does not appear to be involved in the PDGF receptor-mediated effects, whereas activation of phosphatidylinositol (PI) 3-kinase is a crucial event in all pathways leading to stimulation of glucose transport. [Diabetologia (1996) 39: 1432–1439]

Keywords Insulin, glucose transport, tyrosine kinase, 3T3-L1 cells, PDGF receptor.

Received: 14 December 1995 and in final revised form: 26 August 1996

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Abbreviations: PDGF, Platelet derived growth factor; PDGF-R, platelet derived growth factor receptor; IGF-I, insulin-like growth factor I; IR, insulin receptor; IRS-1, insulin receptor substrate 1; JAK, Janus kinase; STAT, signal transducer and activator of transcription; GLUT1, erythrocyte-type glucose transporter 1; GLUT4, adipose/muscle-type glucose transporter; IL-6, interleukin 6; sIL-6R, soluble receptor of interleukin 6; IL-4, interleukin 4; KRH, Krebs-Ringer Hepes buffer; BSA, bovine serum albumin; EGF, epidermal growth factor; APRF, acute-phase response factor.

Several growth factors exert their mitogenic effect through the intrinsic tyrosine kinase activity located at the intracellular domain of their receptors [1]. The common event in the signal transduction of these receptors is the formation of a multimeric complex comprising the phosphotyrosine domains of the receptor and SH2/SH3 domains of effector or adapter proteins [2–4]. The phosphorylated platelet derived growth factor (PDGF) receptor appears to directly interact with SH2 domains in phosphatidylinositol (PI) 3-kinase or other proteins [5, 6]. Similarly, phosphorylated epidermal growth factor (EGF)-receptors have been shown to form a complex including the SH2/SH3 adapter Grb-2 and the GTP exchange factor Sos-1 which subsequently activates Ras [7]. In

contrast, insulin receptors phosphorylate substrates (IRS-1 and IRS-2) on tyrosine residues that appear to constitute the docking sites for SH2 domains in other proteins, e.g. PI 3-kinase [8, 9].

In mammals, there is a clear functional distinction between insulin receptors and other members of the protein tyrosine kinase family. Whereas insulin receptors regulate metabolic pathways, e.g. the transport of glucose in muscle and adipose tissue, all other tyrosine kinase receptors so far identified appear to control cell growth. Consequently, the question arises whether this specificity reflects a heterogeneity of the signalling pathways, or whether it is the expression and the selectivity of the receptors alone that determine the cellular responses. If the second possibility was true, one would expect that other tyrosine kinase receptors could mimick the metabolic effects of insulin in cells equipped with an insulin-sensitive glucose transport *and* the respective growth factor receptor.

We have previously employed 3T3-L1 preadipocytes in order to address this question [10]. This cell acquires a moderate insulin sensitivity of glucose transport at confluence, and a marked insulin sensitivity after differentiation to an insulin-like phenotype [11–14]. A comparison of the effects of insulin and IGF-I on glucose transport in the differentiated cells indicated that the IGF-I receptor, the closest relative of the insulin receptor, is capable of stimulating the same response of glucose transport as the insulin receptor [10]. In contrast, more recent work by others has suggested that the PDGF receptor lacks the potential to exert an insulin-like effect on glucose transport in 3T3-L1 adipocytes [15–17].

Recently, it was shown that IRS-1, the main substrate of the insulin receptor kinase, is phosphorylated in response to IL-4, suggesting that the pathways controlled by cytokines may interact with those controlled by insulin and growth factors [18, 19]. In the present study we investigated the effects of interleukin 6 (IL-6), interferon-γ, and IL-4 on glucose transport. IL-6 was chosen because it can stimulate cells lacking IL-6 receptors through an exogenously added soluble receptor protein which binds to the ubiquitously expressed signal transducer of the IL-6 receptor, gp130 [20, 21]. IL-6 and interferon-γ activate the transcription factors acute-phase response factor (APRF)/Stat3 or Stat1, respectively, through the tyrosine kinases Jak1, Jak2 and Tyk2 [22, 23], whereas IL-4 activates Stat6 through Jak1 and Jak3 [23]. Our data indicate that IL-6 and interferon-γ receptors lack the potential to stimulate glucose transport in both phenotypes of the 3T3-L1 cells. PDGF receptors, in contrast, produce a full insulin-like effect in confluent 3T3-L1 fibroblasts which differs from that produced by insulin by the absence of any detectable IRS-1 phosphorylation. In addition, we show that the incomplete stimulation of glucose transport by PDGF in the differentiated cells is paralleled by a markedly reduced expression of PDGF-receptors.

Materials and methods

Cell culture. 3T3-L1 fibroblasts were obtained from the American Type Culture Collection (Rockville, Md., USA) and were grown and differentiated as described previously [10].

Assay of glucose transport. Confluent 3T3-L1 fibroblasts (day 7 after plating) or differentiated cells (day 9 after induction of differentiation) were washed with Krebs-Ringer Hepes buffer (KRH) supplemented with 0.2 % bovine serum albumin (BSA), and were incubated for 2 h in serum free culture medium containing 1 % BSA. Thereafter, cells were washed two times with KRH containing 0.2 % BSA and were incubated for 20 min in this buffer supplemented with 1 % BSA (basal) and the indicated concentrations of hormones. Recombinant IGF-I was a gift from Ciba-Geigy (Basel, Switzerland); PDGF-AA, PDGF-BB, and IL-4 were purchased from Bachem (Bubendorf, Switzerland). IL-6 and the soluble IL-6 receptor were prepared as described previously [24]. Recombinant rat interferon-γ was a gift from Dr. M.L. Lohmann-Matthes, Abteilung Immunologie, Fraunhofer Institut, Hannover. Glucose transport activity was determined with 2-[^{3}H]deoxyglucose as described previously [10].

Preparation of nuclear extracts and gel retardation assay. 3T3-L1 fibroblasts and adipocytes were kept for 2 h in serum-free culture medium (1 % BSA), and were stimulated for 15 min as indicated with IL-6/sIL-6R, interferon-γ or with insulin. Nuclear extracts were prepared as described [25]. Gel retardation assays were performed according to a modification of a published procedure [26]. A ^{32}P-labelled synthetic oligonucleotide (5′-GATCTAGCATTTCCCGTAAATCCCTCCCG-3′) corresponding to SIEm67 (*sis*-inducible element of the c-*fos* promoter) was used as a probe. Nuclear extracts (5 μg of protein) were incubated with about 10 fmol (5,000 cpm) of probe in gel shift incubation buffer [10 mmol/l Hepes, pH 7.8, 1 mmol/l EDTA, 5 mmol/l magnesium chloride, 10 % (v/v) glycerol, 1 mmol/l dithiothreitol, 0.07 mmol/l phenylmethylsulfonyl fluoride, 0.1 % BSA, 0.05 mg/ml poly (dIdC) (Pharmacia, Freiburg, Germany)] for 10 min at room temperature. The DNA-protein complexes formed were then separated by native gel electrophoresis on a 5 % polyacrylamide gel containing 7.5 % glycerol, 22 mmol/l Tris borate and 0.5 mmol/l EDTA, pH 8.3.

Preparation of cell lysates and immunoprecipitation. Undifferentiated or differentiated 3T3-L1 cells were stimulated with the indicated agents, and were lysed for 5 min in a boiling SDS-buffer (20 mmol/l Tris, pH 7.4, 1 % SDS). The lysates were then sonicated briefly for 15 s. Approximately 250 μl of the lysates (1 mg of total protein) were incubated for 2 h with 5 μg of phosphotyrosine antibody (PY20, Transduction Laboratories, Lexington, Ky., USA) in a buffer containing 20 mmol/l Tris, pH 7.4, 150 mmol/l sodium chloride, 0.2 mmol/l phenylmethylsulfonyl fluoride and 1 % Triton X-100 (4 °C). Immunocomplexes were adsorbed to protein A sepharose (Pharmacia), washed three times with buffer containing 20 mmol/l Tris pH 7.4, 150 mmol/l sodium chloride and 0.1 % Triton X-100, two times with the same buffer containing 0.05 % Triton X-100 and eluted with electrophoresis sample buffer. The samples were separated by SDS-PAGE and transferred onto nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany) with a tank-blot apparatus (Pharmacia).

Membranes were incubated for 2 h at room temperature with the phosphotyrosine antibody PY20 at a dilution of 1:1000 or with the antiserum against IRS-1 (anti-Rat carboxy-terminal IRS-1, UBI, Lake Placid, N.Y., USA) at a dilution of 1:200. Bound immunoglobulin was detected with ^{125}I-protein A (Amersham-Buchler, Braunschweig, Germany).

Preparation of RNA and synthesis of cDNA. Total RNA was prepared by centrifugation on cesium chloride as described previously [10]. First-strand cDNA was synthesized from adipocyte RNA with murine reverse transcriptase (First-strand cDNA synthesis-kit, Pharmacia) by oligo-dT priming.

Northern blot analysis. Denatured samples of total RNA (20 μg) were separated by electrophoresis on 1 % agarose gels and transferred on to nylon membranes (Hybond N$^+$; Amersham-Buchler). Before transfer, gels were stained with ethidium bromide in order to ascertain that equal amounts of total RNA had been separated. cDNA probes corresponding with the subdomains VI to IX of the catalytic domain of PDGF receptors were generated by PCR (PDGFα-receptor, bp 2635–2837, GenBank accession no. M84607; PDGFβ-receptor, bp 2620–2847, accession no. X04367) with cDNA from differentiated cells. The probes were labelled by random oligonucleotide priming with the Klenow fragment of DNA polymerase I and [^{32}P]CTP. The nylon membranes were hybridized at 42 °C, and blots were washed three times with 0.12 mol/l NaCl/0.012 mol/l sodium citrate/0.1 % SDS at 55 °C.

Results

Comparison of the effects of insulin, IL-6 and interferon-γ on glucose transport in 3T3-L1 cells. The cytokine IL-6 stimulates activation of the transcription factor APRF/Stat3 in a number of cells via a JAK/STAT pathway [22, 27]. In order to compare this signalling pathway with that of tyrosine kinase receptors, we characterized the effects of insulin and IL-6 on APRF/Stat3-activation and glucose transport in 3T3-L1 cells. 3T3-L1 fibroblasts and adipocytes were treated with insulin or with human IL-6. Since we supposed that 3T3-L1 cells have only a low number of IL-6 receptors, the soluble IL-6 receptor (sIL-6R) was added together with IL-6 in separate samples. Proteins were extracted from the nuclei and analysed in gel retardation assays. As is illustrated in Figure 1 (upper panel), a retarded band appeared in extracts from 3T3-L1 fibroblasts treated with IL-6. This band represents a complex of the DNA probe with the activated homodimer of APRF and its hetero-dimer of APRF and Stat1; their mobilities were identical with those of the complexes formed by the APRF hetero- and homodimers in HepG2 cells stimulated with IL-6 (control). Furthermore, the addition of soluble IL-6 receptor to the medium markedly enhanced the effect of IL-6. This finding indicates that IL-6 receptors are limiting for the APRF activation in 3T3-L1 cells. In contrast to IL-6, insulin failed to stimulate APRF-activation in both fibroblasts and adipocytes.

The lower panel of Figure 1 illustrates the effects of IL-6 and insulin on glucose transport (2-deoxyglucose uptake). Insulin exerted a moderate (four-fold) stimulation of the 2-deoxyglucose uptake in confluent fibroblasts. As was previously shown [14, 13], this effect is due to the translocation of GLUT1 from an intracellular pool which is formed during growth of cells to confluence but prior to the initiation of differentiation. In differentiated cells, the anticipated large increase of glucose transport activity was observed (16-fold, note also the different scales of the ordinate). In contrast, glucose transport was unresponsive to IL-6 or the combination of IL-6 with its soluble receptor.

The cytokine interferon-γ stimulates Jak1 and Jak2 through its receptor independent of the gp130 subunit of the IL-6 receptor. This effect of interferon-γ was shown in 3T3-L1 cells by a marked activation of Stat1 and a weak activation of APRF/Stat3 as

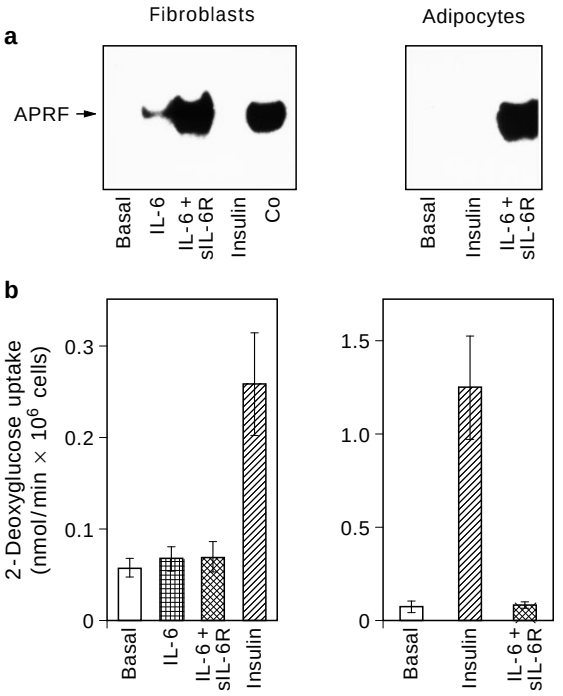

Fig. 1 a, b. Effects of insulin and IL-6 on APRF/Stat3 activation and glucose transport in 3T3-L1 cells. **a**) Activation of APRF in 3T3-L1 fibroblasts and adipocytes by IL-6 in the presence or absence of soluble IL-6 receptor (sIL-6R), and lack of effect of insulin. 3T3-L1 cells were treated for 15 min with human IL-6 (200 units/ml) and sIL-6R (400 ng/ml) or insulin (250 nmol/l). Nuclear extracts were prepared and subjected to a gel retardation assay as described, using 5 μg of nuclear protein per lane and a ^{32}P-labelled SIEm67 (*sis*-inducible element of the c-*fos* promoter) probe. As a control (Co) 5 μg of nuclear extract from HepG2 cells treated with IL-6 were used. **b**) Effect of insulin on glucose uptake in 3T3-L1 fibroblasts and 3T3-L1 adipocytes, and lack of effect of IL-6/sIL6-R. 3T3-L1 cells were treated for 20 min without or with human IL-6 (200 U/ml), IL-6 and sIL-6R (400 ng/ml) or insulin (250 nmol/l). Glucose uptake was determined with tritiated 2-deoxyglucose as described in Materials and methods. Data represent means ± SEM of three independent experiments, each performed with duplicate samples

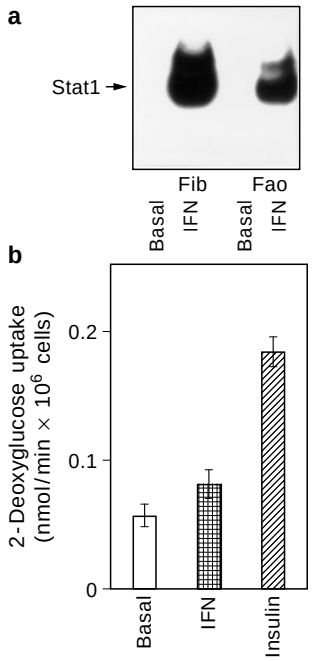

Fig. 2. Effects of interferon-γ (IFN) and insulin on Stat1 activation and glucose transport in 3T3-L1 cells. **a**) Confluent 3T3-L1 cells (Fib) or Fao cells (Fao) were treated for 15 min with rat interferon-γ (500 and 250 units/ml, respectively), and nuclear extracts were prepared and subjected to a gel retardation assay as described. **b**) Confluent 3T3-L1 cells were treated for 20 min with or without rat interferon-γ (500 U/ml) or insulin (250 nmol/l). Glucose uptake was determined with tritiated 2-deoxyglucose as described in Materials and methods. Data represent means ± SEM of three independent experiments

demonstrated in the gel retardation assay (upper panel of Fig. 2). In contrast, interferon-γ failed to produce an insulin-like stimulation of glucose transport in confluent fibroblasts (Fig. 2, lower panel) or adipocytes (data given in next paragraph).

IL-6 and interferon-γ are believed to produce STAT activation through the tyrosine kinases Jak1 and Jak2 and Tyk2, whereas other interleukins, e.g. IL-4, appear to require Jak3 [23]. Thus, experiments with IL-4 were performed. Like IL-6 and interferon-γ, IL-4 failed to stimulate an insulin-like response of glucose transport in both fibroblasts and adipocytes (Confluent fibroblasts: basal uptake rate, 0.042 ± 0.005; IL-4, 0.058 ± 0.009; insulin, 0.189 ± 0.015. Adipocytes: basal uptake rate, 0.064 ± 0.005; IL-4, 0.083 ± 0.002; interferon-γ, 0.089 ± 0.004; insulin, 0.713 ± 0.018 nmol/(min × 10^6 cells).

Comparison of the effects of insulin, IGF-I, IL-6, IL-4, and interferon-γ on IRS-1 phosphorylation in 3T3-L1 cells. In order to compare the effects of insulin, IGF-I, IL-6, IL-4 and interferon-γ on tyrosine phosphorylation of the insulin receptor substrate IRS-1, 3T3-L1 cells were treated with the agents, lysed, and the lysates were incubated with the monoclonal antibody (PY20) against phosphotyrosine. The immunoprecipitates were analysed by Western blotting with the same antibody. As is illustrated in Figure 3 a, insulin and IGF-I stimulated the tyrosine phosphorylation of a 160 kDa protein in both fibroblasts and adipocytes. In parallel immunoblots with a specific antiserum (data not shown), this band was identified as IRS-1. In the fibroblasts, the magnitude of the phosphorylation in response to insulin was identical to that elicited by IGF-I. In the adipocytes, insulin produced an approximately five-fold higher tyrosine phosphorylation of IRS-1 than IGF-I (quantitation by laser densitometry: basal, 0.052 ± 0.026 arbitrary units; insulin, 3.03 ± 0.23; IGF-I, 0.71 ± 0.14; means ± SEM of three experiments). The weaker effect of IGF-I appears to correspond with the lower number of IGF-I receptors in differentiated 3T3-L1 cells [10]. Glucose transport stimulation by insulin and IGF-I was assayed in parallel experiments. As anticipated [10], both hormones produced an essentially identical stimulation of 2-deoxyglucose uptake (basal fibroblasts, 0.046 ± 0.01; insulin, 0.213 ± 0.05; IGF-I, 0.243 ± 0.03; basal adipocytes, 0.068 ± 0.001; insulin 1.36 ± 0.18; IGF-I, 1.25 ± 0.07 nmol/(min × 10^6 cells), means ± SEM of three experiments). Thus, a submaximal IRS-1 phosphorylation as seen with IGF-I appears to be sufficient to elicit a full stimulation of glucose transport.

In contrast to the effects of insulin and IGF-I on IRS-1 phosphorylation, IL-6/sIL-6R, interferon-γ and IL-4 failed to stimulate any detectable IRS-1 phosphorylation in 3T3-L1 adipocytes (Fig. 3 b). It should be noted that the cytokines produced a very weak phosphorylation of an unidentified band which migrates with a slightly higher electrophoretic mobility (155 kDa) than IRS-1 (160 kDa). In addition, phosphorylation of two other unidentified bands (116 and 135 kDa) was detected in adipocytes, but was not altered by any of the hormones or cytokines.

Differential expression of PDGF-receptors in 3T3-L1 cells and effects of PDGF on glucose transport. It has previously been shown that PDGF stimulates glucose transport in differentiated 3T3-L1 cells to a much lower extent than insulin [15–17]. In contrast, PI 3-kinase appeared fully stimulated by PDGF in 3T3-L1 adipocytes [16, 17]. In order to detect possible differences between fibroblasts and adipocytes in the abundance of receptors and/or the signal transduction, we studied the effects of PDGF on the receptor autophosphorylation and on tyrosine phosphorylation of receptor substrates. As is illustrated in Figure 3 c, PDGF produced a strong tyrosine phosphorylation of a 190 kDa protein in 3T3-L1 fibroblasts, corresponding with the PDGF receptor. In contrast, autophosphorylation of the PDGF receptor in the adipocytes was only 20 % (laser densitometry: fibroblasts, 0.98 ± 0.16 arbitrary units; adipocytes 0.20 ± 0.06; means ± SEM) of that seen in the fibroblasts. As anticipated, insulin markedly stimulated tyrosine phosphorylation of

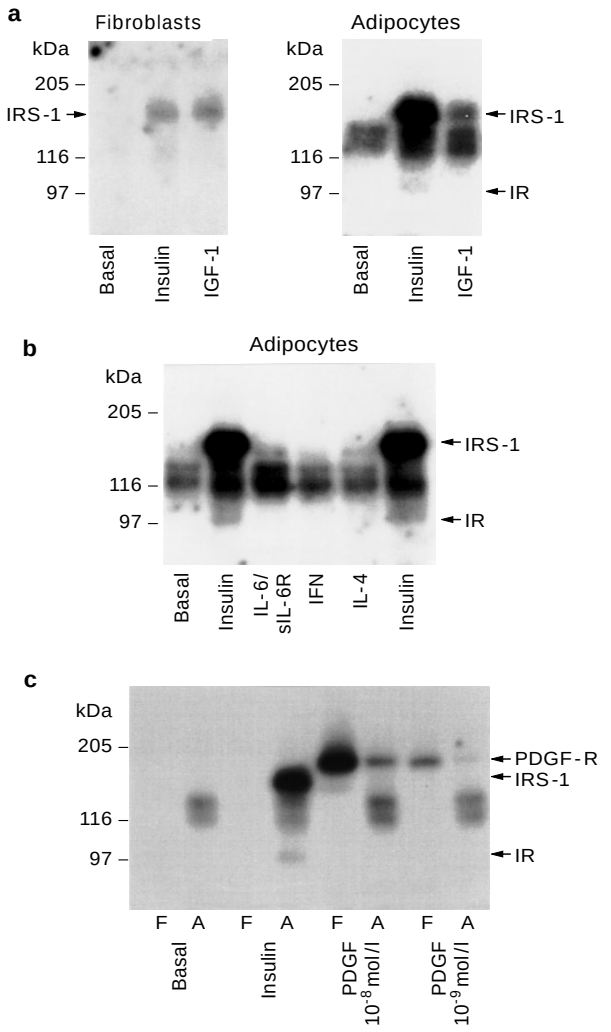

Fig. 3 a–c. Comparison of the effects of insulin, IGF-I, IL-6, interferon-γ, IL-4 and PDGF on IRS-1 phosphorylation in 3T3-L1 cells. **a**) IRS-1 phosphorylation in 3T3-L1 fibroblasts and adipocytes in response to insulin and IGF-I. The cells were treated for 2 min without or with insulin (250 nmol/l) or IGF-I (10 nmol/l) and lysed in SDS-buffer. Phosphoproteins in the lysates were immunoprecipitated with antibody against phosphotyrosine (PY20). Immunoprecipitates were separated by SDS-PAGE, transferred on to nitrocellulose and probed with PY20. In separate immunoblots with specific antiserum (not shown), the identity of IRS-1 was confirmed. **b**) Lack of effect of IL-6, interferon-γ (IFN), and IL-4 on IRS-1 phosphorylation in 3T3-L1 adipocytes. Differentiated 3T3-L1 cells were treated for 15 min with insulin (250 nmol/l), IL-6 (200 U/ml) plus sIL-6R (400 ng/ml), interferon-γ (500 U/ml) or IL-4 (1000 U/ml) and lysed in SDS-buffer. Phosphoproteins in the lysates were isolated and detected as described above. **c** Effects of insulin and PDGF on IRS-1 phosphorylation in 3T3-L1 cells. Undifferentiated (F, fibroblasts) and differentiated (A, adipocytes) 3T3-L1 cells were incubated for 2 min without or with insulin (250 nmol/l) or PDGF-BB (10^{-8} mol/l, 10^{-9} mol/l) and lysed in SDS-buffer. Phosphoproteins in the lysates were isolated and detected as described above

IRS-1 (160 kDa) in the adipocytes. In contrast, PDGF failed to stimulate any detectable IRS-1 phosphorylation in either fibroblasts or adipocytes.

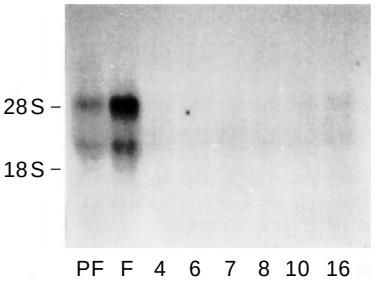

Fig. 4. Time course of expression of the PDGF-receptor during growth and differentiation of 3T3-L1 cells to the adipocyte-like phenotype. Total RNA from the indicated cells was hybridized with the PDGF-α receptor-specific probe. PF, preconfluent cells on day 4 after plating; F, confluent fibroblasts on day 7. Numbers designate the days after induction of the differentiation. All lanes contained equal amounts of RNA as judged from ethidium bromide staining and hybridization with other probes e.g., ARL1, ARL4 and GLUT4 [36]

The data on the PDGF-stimulated receptor autophosphorylation (Fig. 3) suggested a differential expression of the PDGF receptors in 3T3-L1 cells. Thus, a Northern blot analysis of PDGF receptor mRNA in 3T3-L1 cells during differentiation was carried out with probes specific for either isotype. In the Northern blot obtained with a probe specific for the PDGF-α receptor (Fig. 4), two transcripts were detected in growing, pre-confluent cells (PF, day 4 after plating). Two days after confluence (F, day 7 after plating), the intensity of the signals from both transcripts was approximately doubled. Thereafter, the mRNA levels of PDGF-α receptors decreased to nearly undetectable levels at day 4 after initiation of differentiation. With a specific probe for the PDGF-β receptor, considerably weaker signals were detected in fibroblasts which decreased to undetectable levels in the adipocytes (data not shown).

In order to compare the differences in the PDGF receptor expression in fibroblasts and adipocytes with those in glucose transport stimulation, the effects of PDGF-AA and PDGF-BB on glucose transport activity were studied with the 2-deoxyglucose uptake method. In confluent 3T3-L1 fibroblasts, PDGF-AA as well as PDGF-BB produced an approximately six to eight-fold stimulation of 2-deoxyglucose uptake (Fig. 5). Half-maximal stimulation was observed at a concentration range between 0.1 and 1 nmol/l. The magnitude of the stimulatory effect of PDGF-AA was similar to that of insulin; that of PDGF-BB was even greater than that of insulin. In differentiated cells (note the different ordinate scale), insulin produced an approximately 25-fold stimulation of the 2-deoxyglucose uptake (Fig. 5, right panel). In contrast, PDGF-AA and PDGF-BB produced a much lower stimulation which amounted to only a small portion of that seen with insulin. Again, the effect of PDGF-BB was clearly higher than that of PDGF-AA.

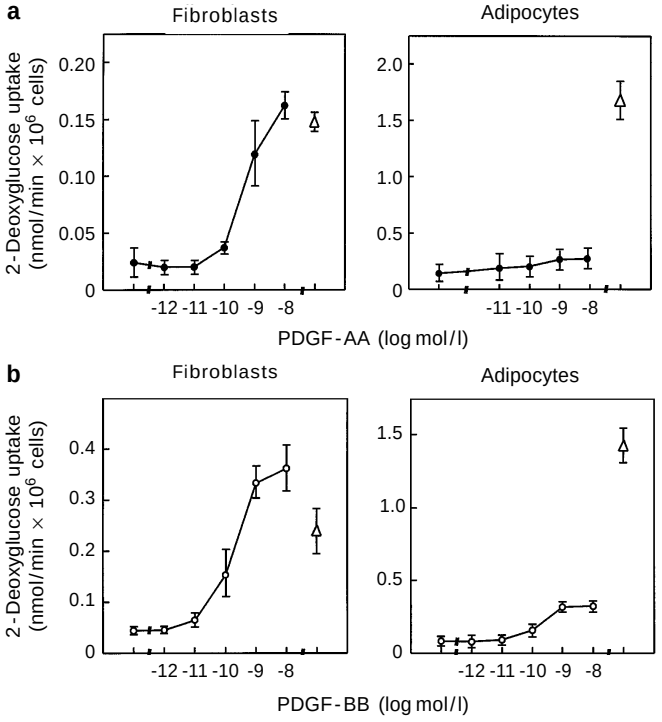

Fig. 5 a, b. Effects of PDGF-AA (a) and PDGF-BB (b) on glucose uptake in 3T3-L1 cells. 3T3-L1 cells (left panels, fibroblasts; right panels, adipocytes) were incubated for 20 min with the indicated concentrations of PDGF-AA (●), PDGF-BB (○) or insulin (250 nmol/l, △). Glucose uptake was determined with tritiated 2-deoxyglucose as described in Materials and methods. Data represent means ± SEM of at least three independent experiments, each performed with duplicate samples

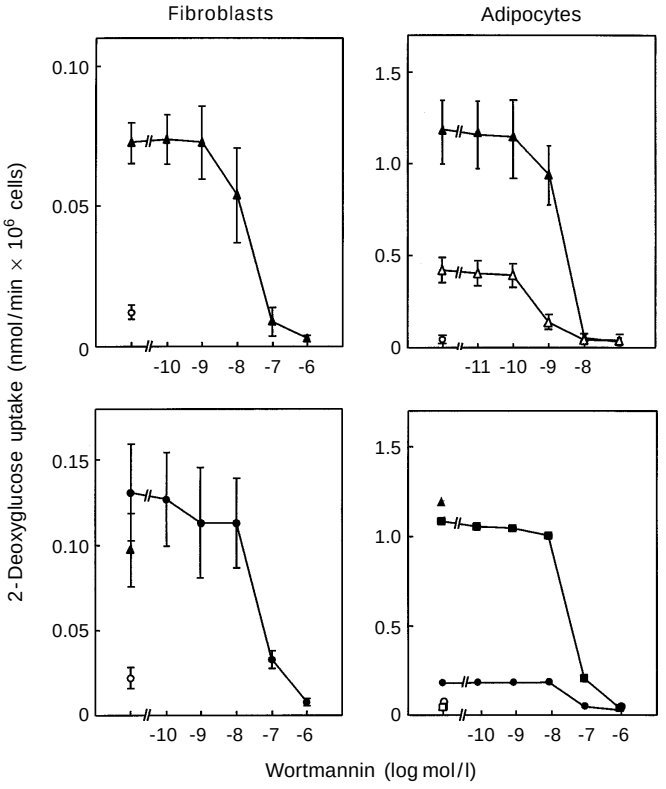

Fig. 6. Inhibition of insulin, IGF-I, and PDGF-stimulated glucose uptake by Wortmannin in 3T3-L1 cells. 3T3-L1 cells (left panels, fibroblasts; right panels, adipocytes) were incubated for 30 min with the indicated concentrations of Wortmannin and insulin (250 nmol/l ▲; 3 nmol/l △), PDGF-BB (fibroblasts, 10^{-9} mol/l; adipocytes; 10^{-8} mol/l ●) and IGF-I (10^{-8} mol/l, ■) or without hormone (○ □). Glucose uptake was determined with tritiated 2-deoxyglucose as described in Materials and methods. Data represent means ± SEM of three experiments or means of duplicate samples from a representative experiment (lower right panel)

Effects of Wortmannin on glucose transport activity stimulated by insulin, IGF-I, and PDGF. PI 3-kinase is stimulated by insulin and has been suggested to mediate the effect of insulin on glucose transport [28, 29]. Furthermore, the specific PI 3-kinase inhibitor Wortmannin is known to inhibit insulin-stimulated translocation of GLUT1 and GLUT4 in 3T3-L1 adipocytes [30]. Based on the assumption that PI 3-kinase plays a crucial role in all signalling pathways leading to an insulin-like transporter translocation, we anticipated that Wortmannin would inhibit all stimulatory effects of the growth factors. Indeed, as is shown in Figure 6, Wortmannin inhibited 2-deoxyglucose uptake stimulated by insulin, IGF-I, and PDGF in a concentration-dependent manner. The IC_{50} values of Wortmannin were similar (approximately 20 nmol/l) for the stimulation of glucose transport by insulin (250 nmol/l), IGF-I and PDGF. Interestingly, the IC_{50} value of Wortmannin was lower at low insulin concentrations (3 nmol/l; upper right panel of Fig. 6). The latter finding strongly suggests a competitive component of the effects of insulin and Wortmannin, and further strengthens the previous conclusion [28, 29] that the effect of insulin on 2-deoxyglucose uptake is mediated by activation of PI 3-kinase.

Discussion

Signalling pathways interact through a complex network of regulation which modulates the cellular response to different, simultaneously acting stimuli. Recently, cross-talk between a JAK/STAT pathway and components of the tyrosine kinase receptor pathways has been demonstrated [19, 18]. It was shown that IRS-1, the primary substrate and adaptor of the insulin receptor, plays an essential role in the mitogenic effects of IL-4. Furthermore, Map-kinase appears to mediate not only effects controlled by tyrosine kinase receptors but also the cellular response to interferons [31, 32]. Thus, based on the hypothesis that JAK kinases can phosphorylate IRS-1, it was conceivable that glucose transport is stimulated by receptors triggering JAK/STAT pathways. In order to test this assumption, we studied the effects of IL-6 and interferon-γ, which activate different transcription factors (Stat3 and Stat1) through related pathways involving Jak1, Jak2 and Tyk2. In addition, experiments were

performed with IL-4 which activates Stat6 through Jak1 and Jak3.

The present data indicate that both IL-6 and interferon-γ failed to increase 2-deoxyglucose uptake rates or stimulate IRS-1 phosphorylation in 3T3-L1 cells. Conversely, insulin was without detectable effect on APRF/Stat3 or Stat1 activation as demonstrated in the gel retardation assays. Thus, our data demonstrate a lack of cross-talk between the IL-6 or interferon-γ controlled JAK/STAT pathways, and the insulin/IGF-I receptor pathway in 3T3-L1 cells. It has to be noted, however, that this conclusion is restricted to the JAK isoforms which associate with the IL-6 receptor/gp130 complex or the interferon-γ receptor (Jak1, Jak2, Tyk2). The data cannot rule out that other JAK isoforms are capable of triggering the pathway that initiates translocation of glucose transporters. Furthermore, we cannot discount the possibility that the failure of IL-4 to stimulate glucose transport is due to a lack of IL-4 receptors or the Jak3 kinase in 3T3-L1 cells.

The present data indicate that both PDGF-AA and PDGF-BB exert the full effect of insulin in undifferentiated fibroblasts (six – eight-fold stimulation), but failed to stimulate more than 20 % of the maximal effect of insulin in adipocytes. Thus, the data confirm previous reports indicating differences in the signalling potential of insulin and PDGF receptors in 3T3-L1 cells [33, 15–17]. The data are compatible with the conclusion that PDGF receptors produce a full insulin-like translocation of GLUT1, which is predominant in fibroblasts, but not of GLUT4, which is present only in the adipocytes. This conclusion has previously been proposed based on findings with the impermeant bis-mannose photolabel [15]. Furthermore, since PDGF failed to stimulate IRS-1 phosphorylation in both phenotypes of the 3T3-L1 cell, it might be speculated that IRS-1 phosphorylation is required for insulin-stimulated translocation of GLUT4 but not of GLUT1.

We have shown here that levels of mRNA and protein of PDGF receptors are markedly decreased after differentiation of 3T3-L1 cells. Thus, the possibility has to be considered that the failure of PDGF to stimulate the full insulin response in adipocytes is due to this loss of PDGF receptors. Previous reports suggesting that PDGF produces a full stimulation of PI 3-kinase in adipocytes [16, 17] appear to argue against this conclusion. We cannot fully discount the possibility that the differentiation-dependent loss of receptors is specific for the 3T3-L1 clone used in our laboratory. However, the previous conclusion [16, 17] that PI 3-kinase is necessary, but not sufficient for the insulin-stimulated translocation of GLUT4 is not incompatible with our data. It is conceivable that the reduced number of receptors is sufficient for full PI 3-kinase activation, but insufficient for the presumed additional signalling event(s) required for GLUT4 translocation.

Previous studies with Chinese hamster ovary (CHO) cells overexpressing both PDGF-receptors and GLUT4 indicate that the PDGF-receptor is capable of mediating a stimulation of glucose transport [34]. Furthermore, it has recently been shown that epidermal growth factor (EGF)-receptors when overexpressed in 3T3-L1 adipocytes [35] can produce the full effect of insulin on glucose transport. Together with our data showing the full effect of PDGF on glucose transport in the 3T3-L1 fibroblasts, these data indicate that the receptors for insulin, PDGF and EGF elicit similar, if not identical cellular responses, probably by virtue of similar and in part identical transduction mechanisms. Consequently, as far as these receptors are concerned, the quality and specificity of a cellular response appears to be determined by the hormone binding domain of the receptor rather than its intracellular domain and/or the signalling cascade.

Acknowledgements. This study was supported by grants from the Deutsche Forschungsgemeinschaft (Jo 117/6–2 and SFB 351).

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