Simultaneous redistribution of mannose 6-phosphate and transferrin receptors by insulin-like growth factors and phorbol ester

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Insulin-like growth factors I and II (IGF-I and IGF-II) and phorbol ester are known to induce in fibroblasts a rapid redistribution of mannose 6-phosphate (M6P)/IGF II-receptors to the cell surface. We compared the redistribution of the M6P/IGF-II receptor with that of the 46 kDa M6P receptor (MPR46) and of receptors for transferrin, low-density lipoprotein (LDL) and epidermal growth factor (EGF) in human fibroblasts under the influence of these effectors. None of the effectors altered the surface expression of receptors for LDL or EGF, which are predominantly located at the cell surface. IGF-I, IGF-II and phorbol ester increased the surface expression of the M6P/IGF-II receptor and of MPR46. The concentration of the transferrin receptor at the cell surface was increased only by IGF-I and IGF-II, with similar kinetics as for the M6P/IGF-II receptor, suggesting that the same mechanism causes redistribution. The increased surface expression of M6P receptors was accompanied by an increased uptake of receptor ligands. The number of transferrin receptors did not correlate with iron uptake, although neither the rate nor the extent of transferrin internalization was changed. These results indicate that the redistribution of several endocytic receptors induced by IGF-I, IGF-II and phorbol ester shows selectivity, and that the uptake of receptor ligand may become uncoupled from the surface expression of the receptors via distinct mechanisms.

INTRODUCTION

The surface expression of several endocytic receptors can be modulated by hormones, growth factors, phorbol esters and receptor ligands. For example, the insulin-induced translocation of glucose transporter to the plasma membrane is accompanied by a 3–5-fold increase at the cell surface of receptors for transferrin and α_2 -macroglobulin and of the mannose 6-phosphate/insulin-like growth factor II (M6P/IGF-II) receptor [1–4]. In fibroblasts IGF-I, IGF-II and phorbol ester induce a rapid and transient redistribution of the M6P/IGF-II receptor from internal membranes to the cell surface. This increase in surface expression is accompanied by a parallel increase in the uptake of receptor ligands [5–7].

The purpose of the present study was to investigate whether the cell surface expression of endocytic receptors is modulated by the various effectors in a co-ordinate manner or differentially. We therefore compared the cell surface expression of the two M6P receptors and of the receptors for transferrin, LDL and EGF in human fibroblasts under the influence of IGF-I, IGF-II or phorbol ester.

MATERIALS AND METHODS

Recombinant IGF-I and IGF-II were kindly provided by Dr. W. Märki (Ciba-Geigy, Basel, Switzerland). Epidermal growth factor (EGF) was from Boehringer Mannheim. Low-density lipoprotein (LDL) and lipoprotein-deficient serum were kindly provided by Dr. I. Filipovic (University of Münster, Germany). M6P (sodium salt), human transferrin and phorbol 12,13-dibutyrate (PDBt) were purchased from Sigma. ^{59}Fe , ^{125}I and

^{125}I -labelled sheep anti-mouse Ig antibodies were obtained from Amersham. Monoclonal antibodies against the M6P/IGF-II receptor (2C2) were as described previously [8]. Monoclonal antibodies (21D3) against the 46 kDa M6P receptor (MPR46) were provided by Dr. A. Hille of this institute. Pentamannose 6-phosphate (PMP) was prepared from *Hansenula holstii* phosphomannan provided by Dr. M. Slodki (United States Department of Agriculture, Northern Regional Research Center, Peoria, IL, U.S.A.) and conjugated to BSA (Serva) as described [8]. PMP-BSA, diferric transferrin, LDL and the monoclonal antibodies 2C2 and 21D3 were iodinated by means of Iodogen (Pierce) [8]; EGF was iodinated by the chloramine T procedure [9].

Cell culture

Human skin fibroblasts were maintained in minimal essential medium (MEM; Seromed) containing 5% (v/v) fetal calf serum (Gibco) plus penicillin and streptomycin (Gibco).

Ligand binding

The cells were washed with serum-free medium and incubated for 60 min in MEM containing 0.02 M-Hepes, pH 7.2, and 0.1% BSA (Sigma A-4378; MEM/BSA). Growth factors or PDBt were added and the cells were incubated for 10 and 30 min respectively at 37 °C. Binding of ^{125}I -PMP-BSA was determined as described [8]. Cell-surface-bound ligand was released by incubation with ice-cold Hanks' solution containing 2 mM-M6P. Binding of ^{125}I -labelled diferric transferrin (5 nM; 4 $\mu\text{Ci}/\mu\text{g}$) and ^{125}I -EGF (0.5 nM; 110 $\mu\text{Ci}/\mu\text{g}$) was carried out for 4 h at 4 °C. Unbound ligands were removed by washing five times with Hanks' solution, and surface-bound ligands were displaced by two washes with 0.2 M-acetic acid/0.5 M-NaCl at pH 2.5 for 5 min [10]. To determine the binding of ^{125}I -LDL, fibroblasts

Abbreviations used: M6P, mannose 6-phosphate; IGF, insulin-like growth factor; PDBt, phorbol 12,13-dibutyrate; EGF, epidermal growth factor; LDL, low-density lipoprotein; PMP-BSA, pentamannosyl 6-O-phosphate-substituted BSA; MPR46, 46 kDa mannose 6-phosphate receptor; MEM, minimal essential medium.

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were incubated for 48 h in MEM containing 5% (v/v) lipoprotein-deficient serum, followed by incubation for 60 min with MEM/BSA and for 10 or 30 min with effectors. Binding of ^{125}I -LDL (5 $\mu\text{g}/\text{ml}$; 3 $\mu\text{Ci}/\mu\text{g}$ of protein) was carried out for 4 h at 4 °C and the cell-surface-bound ligand was released by incubation with 0.1% trypsin (Boehringer) for 1 h at 4 °C [11]. The cells were solubilized in 1 M-NaOH for determination of the cell protein [12] using BSA as standard. Unspecific binding was defined as binding in the presence of 5 mM-M6P or of a 25–100-fold excess of unlabelled ligand. Unspecific binding accounted for 10–20% of total binding in non-stimulated cells. The washing procedures using M6P or acidic buffer displaced 80–90% of cell-surface-bound ligands.

Ligand internalization

Internalization of ^{125}I -PMP-BSA was examined as described [5]. The internalization of freshly prepared ^{125}I -LDL was determined according to [11]. The cells were incubated for 24 h in MEM containing 5% (v/v) lipoprotein-deficient serum, followed by incubation with ^{125}I -LDL (12.5 $\mu\text{g}/\text{ml}$; 1.5–4 $\mu\text{Ci}/\mu\text{g}$) in the same medium for 1 h at 37 °C. The cells were washed, and cell-surface-bound ^{125}I -LDL was released by incubation for 1 h at 4 °C with 0.1% trypsin in 0.01 M-sodium phosphate buffer, pH 7.4, containing 0.15 M-NaCl. ^{59}Fe -labelled diferric transferrin (6.5×10^4 c.p.m./ μg) was prepared from transferrin and $^{59}\text{FeCl}_3$ [13]. Fibroblasts were incubated in MEM/BSA containing ^{59}Fe -transferrin (1.5×10^4 c.p.m./plate; 230 nM) for 30 min at 37 °C. Cells were then washed and the cell-surface-bound ^{59}Fe -transferrin was released by an acid wash [10]. The cells were solubilized in 1 M-NaOH and the acid-wash-resistant radioactivity was determined with respect to cell protein.

Internalization of receptor-specific ^{125}I -labelled antibodies

Fibroblasts were incubated for 1 h at 37 °C in MEM/BSA containing the ^{125}I -labelled monoclonal antibody 2C2 or 21D3 [$(4-5) \times 10^5$ c.p.m./ml; specific radioactivity 4–14 $\mu\text{Ci}/\mu\text{g}$]. After washing the cells, the cell-surface-associated ^{125}I -labelled antibody was removed by incubating the cells for 1 h at 4 °C with 0.1% Pronase in 0.01 M-phosphate buffered saline, pH 7.4. More than 75% of the cell-associated radioactivity was pronase-resistant, and represents internalized ^{125}I -labelled antibodies. In the presence of a 100-fold excess of unlabelled anti-receptor antibodies the internalization of ^{125}I -antibodies was inhibited by 85%.

Statistical analysis

The experiments were analysed by the Wilcoxon multiple comparison test; $P \leq 0.05$ was considered significant.

RESULTS

Effects of IGF-I, IGF-II and phorbol ester on the surface expression of receptors for M6P, transferrin, LDL and EGF

Previous studies had shown that IGF-I, IGF-II and tumour-promoting phorbol esters induce a rapid and transient redistribution of M6P/IGF-II receptors from internal membranes to the cell surface [5,6]. In this study, we compared the cell surface expression of the receptors for transferrin, LDL and EGF with that of the M6P/IGF-II receptor in order to obtain information about the selectivity of the redistribution. Fibroblasts were incubated for 10 min with 10 nM-IGF-I or -IGF-II and then analysed for the binding of ^{125}I -transferrin, ^{125}I -LDL, ^{125}I -EGF or ^{125}I -PMP-BSA (a high-affinity ligand of the M6P/IGF-II receptor). Whereas IGF-I and IGF-II increased the binding of PMP-BSA 2.0–2.7-fold, binding of LDL and EGF

was not affected, and that of transferrin was increased 1.4–1.6-fold (Table 1). Treatment for 30 min with the phorbol ester PDBt (1 $\mu\text{g}/\text{ml}$), which increased binding of PMP-BSA by 4.1-fold, did not change binding of transferrin and LDL, and decreased binding of EGF to 32% of control. The latter is known to result from protein kinase C-mediated phosphorylation of the EGF receptor, which decreases the affinity for EGF [14].

The increase in PMP-BSA binding is known to be due to a redistribution of M6P/IGF-II receptors, and does not depend on receptor synthesis or on an increase in affinity [5]. The dissociation constant (K_d) and number of binding sites (B_{max}) for transferrin in controls were 1.21 nM and 8.8 ng/mg of cell protein respectively (Table 2). IGF-I and IGF-II did not change the affinity of the transferrin receptor for its ligand.

Simultaneous redistribution of M6P/IGF-II and transferrin receptors

If effectors such as IGF-I and IGF-II mobilize the M6P/IGF-II and transferrin receptors from a common intracellular pool or by the same mechanism, then the modulation of cell surface expression in response to these effectors should display the same time-dependence. Cells were incubated for 2–30 min with 10 nM-IGF-I or -IGF-II. It is apparent from Fig. 1 that the changes in the transferrin- and PMP-BSA-binding sites follow similar kinetics. Maximal redistribution to the cell surface was obtained

Table 1. Effects of growth factors and phorbol ester treatment on binding of ^{125}I -PMP-BSA, ^{125}I -transferrin, ^{125}I -LDL and ^{125}I -EGF

Fibroblasts were stimulated for 10 min at 37 °C with growth factors or pretreated with phorbol ester for 30 min prior to binding of ^{125}I -labelled ligand at 4 °C. All values are expressed as percentage of control (means $\pm$ S.D.; n is given in parentheses). Controls bound 0.15 ± 0.10 ng of ^{125}I -PMP-BSA/mg of cell protein; 5.02 ± 2.79 ng of ^{125}I -transferrin/mg; 32.8 ± 19.8 ng of ^{125}I -LDL/mg and 0.15 ± 0.06 ng of ^{125}I -EGF/mg. Statistically significant differences are indicated by * ($P < 0.05$).

Ligand	Pretreatment ...	^{125}I -Ligand binding (% of control)		
		IGF-I	IGF-II	PDBt
PMP-BSA		$269 \pm 69^*$ (21)	$209 \pm 50^*$ (18)	$412 \pm 87^*$ (5)
Transferrin		$157 \pm 24^*$ (22)	139 ± 17 (20)	105 ± 14 (9)
LDL		114 ± 13 (22)	113 ± 9 (22)	99 ± 13 (10)
EGF		93 ± 9 (10)	92 ± 15 (13)	32 ± 8 (6)

Table 2. Equilibrium binding constant (K_d) and number of binding sites (B_{max}) for transferrin in fibroblasts pretreated with growth factors

Fibroblasts were incubated for 10 min at 37 °C with IGF-I or IGF-II. ^{125}I -Transferrin binding was measured over a concentration range of 0.1 nM–0.1 mM, with K_d and B_{max} values being obtained by Scatchard analysis of the binding data by a non-linear regression method. A, B and C represent independent experiments.

	Expt.	Treatment ...	–	IGF-I	IGF-II
K_d (nM)	A		1.23	1.11	1.00
	B		1.33	–	1.03
	C		1.06	1.12	–
B_{max} (ng/mg of cell protein)	A		5.6	9.8	8.7
	B		8.2	–	14.3
	C		11.8	15.1	–

Fig. 1. Time course of IGF-I- and IGF-II-induced redistribution of M6P/IGF-II receptors and transferrin receptors

Fibroblasts were incubated for 60 min in a serum-free medium prior to the addition of 10 nM-IGF-I (a) or 10 nM-IGF-II (b) for the times indicated. After chilling the cells to 4 °C, endogenous M6P-containing ligands were removed by a M6P wash, followed by binding of ^{125}I -PMP-BSA (○) or ^{125}I -transferrin (●). Control fibroblasts bound 0.12 ng of ^{125}I -PMP-BSA/mg of cell protein and 4.5 ng of ^{125}I -transferrin/mg of cell protein.

Fig. 2. Internalization of prebound ^{125}I -transferrin at 20 °C

Fibroblasts were incubated for 10 min at 37 °C with or without IGF-I prior to binding of ^{125}I -transferrin for 4 h at 4 °C. After washing to remove the unbound transferrin, the cells were either harvested ($t = 0$ min) or warmed to 20 °C by addition of prewarmed MEM/BSA in the absence (●) or the presence (○) of 10 nM-IGF-I. At various time points the cells were chilled to 4 °C and the amount of internalized radioactivity (acid-wash-resistant) was determined and expressed as a percentage of total bound ^{125}I -transferrin.

Table 3. Uptake of ^{125}I -PMP-BSA and ^{59}Fe by fibroblasts

Uptake of ^{125}I -PMP-BSA and ^{59}Fe by fibroblasts was determined in the presence of growth factors or PDBt. Fibroblasts were pretreated with PDBt for 30 min at 37 °C. All values are expressed as percentages of control (means $\pm$ S.D., or both values given where $n = 2$). Controls endocytosed 0.17 ± 0.15 ng of PMP-BSA/30 min per mg of cell protein and 471 ± 42.1 ng of ^{59}Fe -loaded transferrin/30 min per mg of cell protein. The numbers of independent experiments are given in parentheses. Statistically significant differences are indicated by * ($P < 0.05$).

Ligand	Treatment...	Uptake (% of control)		
		IGF-I	IGF-II	PDBt
^{125}I -PMP-BSA		$209 \pm 49^*$ (23)	156 ± 42 (26)	$327 \pm 135^*$ (10)
^{59}Fe		89 ± 9 (6)	102 ± 110 (2)	110 ± 89 (2)

Table 4. Steady-state distribution of ^{125}I -transferrin in control and IGF-I-stimulated fibroblasts at 37 °C

Fibroblasts were incubated with ^{125}I -transferrin in the presence or the absence of IGF-I for 30 min at 37 °C. Cell-surface-associated ^{125}I -transferrin was removed with an acid wash at pH 2.5. The values are given as means $\pm$ S.D. The numbers in parentheses indicate the numbers of independent experiments.

	^{125}I -Transferrin (ng/mg of cell protein)	
	-IGF-I	+IGF-I
Cell surface	4.8 ± 2.5 (12)	8.2 ± 4.0 (13)
Intracellular	12.6 ± 4.3 (12)	13.1 ± 4.2 (13)

within 5 min, and by 30 min about half of the redistributed receptors had disappeared.

Effect of receptor redistribution on ligand internalization

Effector-induced increases in M6P/IGF-II receptors at the cell surface of fibroblasts are paralleled by similar increases in the uptake of PMP-BSA (Table 3). To examine whether the increase

in transferrin receptor expression at the cell surface is paralleled by an increase in iron uptake, the internalization of ^{59}Fe bound to transferrin was measured. In spite of the increased binding of ^{125}I -transferrin by fibroblasts stimulated with IGF-I (see Table 1), no increase in ^{59}Fe uptake was observed (Table 3). We therefore investigated whether the binding of ^{125}I -transferrin is increased at 37 °C in IGF-I-stimulated fibroblasts (Table 4). At steady state, a condition far from the thermodynamic equilibrium [2], the amount of cell-surface-bound ^{125}I -transferrin was increased about 1.8-fold. The data suggest that IGF-I treatment at 37 °C results in an increase in the occupancy of cell surface transferrin receptors. Next we examined whether the extent or rate of internalization of surface-bound ^{125}I -transferrin was altered by growth factors. To facilitate the determination of internalization kinetics, internalization was measured at 20 °C. About 45% of the ^{125}I -transferrin that was bound at 4 °C to control and IGF-I-stimulated cells became internalized upon warming for 30 min to 20 °C (Fig. 2). Internalization kinetics were affected neither by IGF-I (Fig. 2) nor by IGF-II (results not shown). From these data, we conclude that all transferrin receptors at the plasma membrane are internalized with the same kinetics.

Co-ordinate redistribution of M6P receptors

Mammalian cells express a second M6P receptor with an apparent molecular mass of 46 kDa (MPR46). This receptor does not bind IGF-II, and does not bind M6P when expressed at the cell surface (for review see [15]). The distribution of MPR46 had therefore to be measured using an anti-receptor antibody. In fibroblasts the MPR46 number at the cell surface is too low to allow a reliable determination of receptors by the antibody-binding assay, and we thus used a more sensitive endocytosis assay in which the uptake of anti-receptor antibodies during an incubation at 37 °C is measured. The assumption is that a higher uptake of antibodies reflects an increased cell surface expression of receptors. To control this assumption, we measured the uptake of an antibody against the M6P/IGF-II receptor (Table 5). Uptake of this antibody was stimulated 2.4–2.5-fold in cells treated with IGF-I or PDBt. This is comparable with the increase in M6P/IGF-II receptor number at the cell surface (see Table 1) and of the endocytosis of PMP-BSA (see Table 3). Because of the blocking of the IGF-II-binding site by the 2C2 antibody [16], the uptake of this antibody cannot be determined in the presence of IGF-II. Based on these controls, we assume that the 1.7–2.2-fold increase in the uptake of anti-MPR46 antibodies after stimulation

Table 5. Uptake of anti-(M6P receptor) antibodies by fibroblasts

The uptake of ^{125}I -labelled anti-receptor antibodies was determined in the presence of growth factors or PDBt. Fibroblasts were pretreated with PDBt for 30 min at 37 °C. After 1 h incubation at 37 °C, endocytosed antibody was quantified as described in the Materials and methods section. The values are given as means $\pm$ S.D.; the numbers in parentheses represent the numbers of independent experiments. Controls internalized 8.90 ± 3.38 ng of ^{125}I -21D3/h per mg of cell protein and 2.21 ± 0.76 ng of ^{125}I -2C2/h per mg of cell protein. Statistically significant differences are indicated by * ($P < 0.05$). † The uptake of ^{125}I -2C2 in the presence of IGF-II could not be determined because the antibody blocks the IGF-II-binding site [16].

Treatment ...	Endocytosis (% of control)		
	IGF-I	IGF-II	PDBt
MPR46	224 $\pm$ 12* (11)	199 $\pm$ 10* (12)	167 $\pm$ 14* (14)
M6P/IGF-II receptor	235 $\pm$ 25* (9)	–†	251 $\pm$ 48* (9)

with IGF-I, IGF-II or PDBt (Table 5) reflects a redistribution of MPR46 to the cell surface. As a further control, we measured the uptake of anti-(LDL receptor) antibodies. None of the three effectors altered the uptake of anti-(LDL receptor) antibodies (results not shown).

DISCUSSION

In the present study, we have characterized the effects of IGF-I, IGF-II and a tumour-promoting phorbol ester on the cell surface expression of five endocytic receptors. The surface expression of two receptors, the EGF and LDL receptors, was not influenced by any of the effectors. The surface expression of the three other receptors tested was increased by the insulin-like growth factors and, in the case of M6P receptors, by the phorbol ester. In contrast to the EGF and LDL receptors, the two M6P receptors and the transferrin receptor are located predominantly in intracellular membranes [17,18] and can undergo resialylation during recycling, suggesting that they pass the trans Golgi network [19,20]. Similar to the insulin-induced redistribution of glucose transporters and of receptors for transferrin, α_2 -macroglobulin and IGF-II [1–5], the IGF-I, IGF-II- and PDBt-induced redistribution of the two M6P receptors and the transferrin receptor was rapid and independent of protein synthesis. The mechanism by which IGF-I and IGF-II induce the redistribution of M6P and transferrin receptors is not clear. We have previously shown that both growth factors exert their effects through binding to their own receptors [7], which differ in their mode of signal transduction. IGF-I is likely to induce the redistribution through activation of the intrinsic tyrosine kinase activity of the IGF-I receptor [21]. IGF-II can couple the M6P/IGF-II receptor to G₂ proteins [22]. The downstream elements regulated by G₂ are unknown. The stimulation of receptor redistribution by IGF-II in human fibroblasts neither depends on protein kinase C activation [6] nor is accompanied by changes in the formation of inositol trisphosphate or cyclic AMP (H. Damke, K. von Figura & T. Bräulke, unpublished work).

IGF-I and IGF-II both induced a simultaneous redistribution of two M6P receptors and the transferrin receptor in fibroblasts. There are several possibilities to explain the differences between the M6P and transferrin receptors in the extent of redistribution induced by IGF-I or IGF-II. First, it is likely that the initial

distribution of receptors between cell surface and intracellular membranes is a major factor in this difference. Under steady-state conditions, 10 % of the M6P receptors [8] and 30 % of the transferrin receptors (H. Damke, unpublished work) are present at the cell surface. Intracellular M6P receptors are enriched in late endosomes [17], whereas transferrin receptors are concentrated in the early, mildly acid endosomal compartment [23]. Furthermore, the recycling to the trans Golgi network represents a major pathway for M6P receptors due to their transport functions for lysosomal enzymes, whereas transferrin receptors recycle predominantly between the plasma membrane and early endosomes. Inhibitory effects of growth factors on the pathway between endosomes and the Golgi might therefore increase preferentially the endosomal concentrations of M6P receptors recycling to the plasma membrane. Second, IGFs regulate the cell surface expression of endocytic receptors by different mechanisms, e.g. increasing the externalization rate of M6P receptors [7] and decreasing the rate of endocytosis for the transferrin receptor, as observed for IGF-I in A431 cells [2]. However, our results indicating an unchanged rate of transferrin receptor internalization suggest that this possibility is rather unlikely. Third, the cell surface expression of various endocytic receptors is regulated by partly common but also distinct mechanisms. This possibility is confirmed by our data on the effects of phorbol esters which selectively stimulate M6P receptor redistribution. Additionally, this hypothesis includes the direct effects of growth factors on receptor distribution, e.g. by post-translational modification such as protein kinase C-mediated phosphorylation.

The redistribution of endocytic receptors may be important for the growth-promoting effects of IGF-I, IGF-II and phorbol ester by increasing the uptake of nutrients and growth factors. The significance of the redistribution of MPR46 remains elusive, since this receptor does not bind M6P when expressed at the cell surface in normal concentrations at physiological pH, and hence cannot mediate endocytosis of M6P-containing ligands in spite of its cycling between the cell surface and internal membranes [24,25]. In contrast to the M6P/IGF-II receptor, ligand uptake and receptor concentration at the cell surface were uncoupled for the transferrin receptor. In spite of the increased expression of the transferrin receptor in IGF-I- and IGF-II-treated fibroblasts, the transferrin-mediated Fe³⁺ iron uptake was not affected. We could exclude the possibility that IGF-I and IGF-II alter the extent or the kinetics of transferrin receptor internalization. The discrepancy between the effects of IGF-I on transferrin binding versus transferrin-mediated iron uptake may be the result of equilibrium binding at 4 °C recording all transferrin receptors, whereas at 37 °C not all receptors bind and internalize ligands. Therefore our data on the unchanged K_d for diferric transferrin binding at 4 °C after IGF-I and IGF-II treatment could be misleading. In A431 human epidermoid carcinoma and human fibroblast cells a shift in temperature from 0 °C to 37 °C is accompanied by a 26-fold decrease in affinity for diferric transferrin [2] and a 6-fold decrease in affinity for PMP-BSA [8]. Davis *et al.* [2] also showed that IGF-I did not significantly alter the V_{max} of ^{59}Fe accumulation, but decreased the K_m value for diferric transferrin uptake from 57 to 31 nM. The examination of ^{59}Fe uptake under our conditions (230 nM diferric transferrin) could therefore not be suitable to observe IGF effects. Another possibility to explain the unaltered iron uptake is that a greater fraction of transferrin receptors cycles back to the cell surface without releasing the iron into endosomes. Alternatively, iron uptake may be independent of receptor-mediated endocytosis. This process has been proposed in the redox model of iron uptake, which depends only on binding of transferrin by the transferrin receptor [26].

In summary, our study clearly demonstrates that redistribution of receptors by IGF-I, IGF-II and phorbol esters depends on the type of endocytic receptor. IGF-I, IGF-II and phorbol ester may induce the mobilization of these receptors from a common intracellular site, but through distinct mechanisms. Furthermore, by various means the uptake of ligands can become uncoupled from the effector-induced redistribution of endocytic receptors.

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REFERENCES

1. Cushman, S. W. & Wardzala, L. J. (1980) *J. Biol. Chem.* **255**, 4758–4762
2. Davis, R. J., Faucher, M., Racaniello, L. K., Carruthers, A. R. & Czech, M. P. (1987) *J. Biol. Chem.* **262**, 13126–13134
3. Wardzala, L. J., Simpson, J. A., Rechler, M. M. & Cushman, S. W. (1984) *J. Biol. Chem.* **259**, 8378–8383
4. Covera, S., Graver, D. F. & Smith, R. M. (1989) *J. Biol. Chem.* **264**, 10133–10138
5. Bräulke, T., Tippmer, S., Neher, E. & von Figura, K. (1989) *EMBO J.* **8**, 681–686
6. Bräulke, T., Tippmer, S., Chao, H. J. & von Figura, K. (1990) *Eur. J. Biochem.* **189**, 609–616
7. Bräulke, T., Tippmer, S., Chao, H. J. & von Figura, K. (1990) *J. Biol. Chem.* **265**, 6650–6655
8. Bräulke, T., Gartung, C., Hasilik, A. & von Figura, K. (1987) *J. Cell Biol.* **104**, 1735–1742
9. Zapf, J., Walter, M. & Froesch, E. R. (1981) *J. Clin. Invest.* **68**, 1321–1330
10. Haigler, H. T., Maxfield, F. R., Willingham, M. C. & Pastan, J. (1980) *J. Biol. Chem.* **255**, 1239–1241
11. Filipovic, I. & von Figura, K. (1980) *Biochem. J.* **186**, 373–375
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
13. Klausner, R. D., van Renswoude, J., Ashwell, G., Kempf, C., Schechter, A. N., Dean, A. & Bridges, K. R. (1983) *J. Biol. Chem.* **258**, 4715–4724
14. Schlessinger, J. (1988) *Biochemistry* **27**, 3119–3123
15. Kornfeld, S. & Mellman, I. (1989) *Annu. Rev. Cell Biol.* **5**, 483–525
16. Bräulke, T., Causin, C., Waheed, A., Junghans, U., Hasilik, A., Maly, P., Humbel, R. E. & von Figura, K. (1988) *Biochem. Biophys. Res. Commun.* **150**, 1287–1293
17. Griffiths, G., Hoflack, B., Simons, K., Mellmann, I. & Kornfeld, S. (1988) *Cell* **52**, 329–341
18. Lamb, J. E., Ray, F., Ward, J. H. & Kaplan, J. (1983) *J. Biol. Chem.* **258**, 8751–8758
19. Snider, M. D. & Rogers, O. C. (1985) *J. Cell Biol.* **100**, 826–834
20. Duncan, J. & Kornfeld, S. (1988) *J. Cell Biol.* **106**, 617–628
21. Ullrich, A. & Schlessinger, J. (1990) *Cell* **61**, 203–212
22. Murayama, Y., Okamoto, T., Ogata, E. & Nishimoto, J. (1990) *J. Biol. Chem.* **265**, 17456–17462
23. Yamashiro, D. J., Tycko, B., Fluss, S. R. & Maxfield, F. R. (1984) *Cell* **37**, 789–800
24. Stein, M., Zijderhand-Bleekemolen, J. E., Geuze, H., Hasilik, A. & von Figura, K. (1987) *EMBO J.* **6**, 2677–2681
25. Watanabe, H., Grubb, J. M. & Sly, W. S. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 8036–8040
26. Thorstensen, K. & Romslo, I. (1990) *Biochem. J.* **271**, 1–10

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