Insulin and insulin-like-growth-factor-I (IGF-I) receptors in *Xenopus laevis* oocytes

Comparison with insulin receptors from liver and muscle

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Insulin and insulin-like-growth-factor-I (IGF-I) receptors were partially purified from full-grown (stages V–VI) *Xenopus laevis* oocytes by affinity chromatography on wheat-germ agglutinin–agarose. Competitive-binding assays revealed high-affinity binding sites for both insulin and IGF-I ($K_d = 2.5 \times 10^{-10}$ M and 8×10^{-10} M respectively). However, IGF-I receptors were about 15 times more abundant than insulin receptors (22.5×10^{11} versus 1.5×10^{11} /mg of protein). Moreover, comparison of intact and collagenase-treated oocytes showed that most of the insulin receptors were in the oocyte envelopes, whereas IGF-I receptors were essentially at the oocyte surface. Oocyte receptors were composed of α -subunits of ~ 130 kDa and a doublet of β -subunits of 95 and 105 kDa, which both had ligand-induced phosphorylation patterns compatible with IGF-I receptor β -subunits. Accordingly, the receptor tyrosine kinase was stimulated at low IGF-I concentrations [half-maximally effective concentration (EC_{50}) ~ 0.5 – 1 nM], and at higher insulin concentrations ($EC_{50} \sim 20$ – 50 nM). Partially purified glycoproteins from *Xenopus* liver and muscle contained mainly receptors of the insulin-receptor type, with α -subunits of 140 kDa in liver and 125 kDa in muscle, and doublets of β -subunits of 92–98 kDa in liver and 85–94 kDa in muscle. Immunoprecipitation of receptors from oocytes, liver and muscle by receptor-specific anti-peptide antibodies suggested that the β -subunit heterogeneity resulted from the existence of two distinct IGF-I receptors in oocytes and of two distinct insulin receptors in both liver and muscle. In the different tissues, the two receptor subtypes differed at least by their β -subunit C-terminal region.

INTRODUCTION

Insulin and insulin-like growth factor-I (IGF-I) induce pleiotropic responses in many cell types, including activation of metabolic processes such as glucose or amino acid uptake, glycogen synthesis and stimulation of cell growth and differentiation [1]. The receptors for insulin and for IGF-I are highly homologous, sharing similar heterotetrameric structures composed of disulphide-linked α - and β -subunits, the former carrying most or the whole of the ligand-binding site, and the latter having an intrinsic tyrosine kinase activity [2].

Despite their similarities at ligand and receptor levels, insulin and IGF-I are specialized *in vivo*. Whereas insulin mediates mostly metabolic effects, IGF-I acts as a potent growth and differentiation factor [1,3]. However, *in vitro* there is an overlap of biological effects that may result, at least in part, from spill-over of the ligand to the other receptor at high peptide concentrations [4–6].

For the insulin receptor, autophosphorylation of its β -subunits on tyrosine residues is the earliest post-binding event identified so far. However, the molecular basis of post-receptor signalling and of receptor biological specificity is not known [7,8]. A system allowing experimental modulation at post-receptor level would be of great interest to study signal transmission by the insulin and IGF-I receptors. The *Xenopus laevis* oocyte offers the potential of such a system, because it can be micro-injected with relative ease [9], and permits evaluation of interactions between receptors encoded by injected mRNA and the endogenous second-messenger pathways [10]. Moreover, in full-grown G2-

arrested oocytes, insulin and IGF-I induce the re-initiation of the meiotic cycle, a process called maturation [11], and activate glucose uptake in a concentration-dependent manner [12,13]. IGF-I is more potent than insulin in mediating these responses, suggesting that insulin and IGF-I effects are mainly mediated through IGF-I receptors [12,13]. However, the exact molecular identity of oocyte receptors has not been determined so far.

In this study, we have characterized the specific binding of insulin and IGF-I to partially purified receptors from *Xenopus* oocytes. We have also examined their autophosphorylation properties and studied their subunit composition by immunoprecipitation by anti-peptide antibodies followed by SDS/PAGE analysis. Our data show that full-grown oocytes bear two different subtypes of IGF-I receptors, in addition to low amounts of insulin receptors. These receptors have been compared with those present in two other tissues, liver and muscle, where two different subtypes of insulin receptors were found.

EXPERIMENTAL

Materials

Triton X-100, BSA (RIA grade, ref. 7030), poly[Glu-Tyr (4:1)], collagenase (type IA and II) and soybean trypsin inhibitor (STI) were from Sigma, St. Louis, MO, U.S.A. Wheat-germ agglutinin (WGA)–agarose was from BioMakor, Rehovot, Israel. DEAE-Sephacel was from Pharmacia, Uppsala, Sweden. Phenylmethanesulphonyl fluoride and bacitracin were from Serva, Heidelberg, Germany. Aprotinin (Trasylol) was from

Abbreviations used: MBS, Modified Barth saline; IGF-I, insulin-like growth factor-I; STI, soybean trypsin inhibitor; WGA, wheat-germ agglutinin; EC_{50} , half-maximally effective concentration.

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Specia, Paris, France. Pig insulin was from Novo, Copenhagen, Denmark. Human recombinant IGF-I was from Euromedex, Strasbourg, France. Na¹²⁵I was from CEA, Saclay, France. [γ -³²P]ATP (3000 mCi/mmol), [³⁵S]methionine (1000 Ci/mmol) and [³⁵S]cysteine (1000 Ci/mmol) were from Amersham International, Amersham, Bucks., U.K. Frogs were from CRBM (Centre de Recherches de Biochimie Macromoléculaire), Montpellier, France, and were not treated with gonadotropin.

Oocyte isolation

Ovarian lobes were surgically removed from frogs anaesthetized in ice-cold water and transferred to sterile Modified Barth Solution (MBS) as described in [9]. MBS is 15 mM-Hepes/NaOH, pH 7.6, 88 mM-NaCl, 1 mM-KCl, 2.4 mM-NaHCO₃, 0.3 mM-Ca(NO₃)₂, 0.41 mM-MgSO₄, containing 10 μ g of sodium penicillin/ml and 10 μ g of streptomycin sulphate/ml. After rinsing in MBS, stage V–VI oocytes were manually dissected from ovarian lobes. Oocyte envelopes were removed by treatment for 2 h in collagenase IA (2 mg/ml) or in collagenase II (2 mg/ml) and STI (0.4 mg/ml). Healthy oocytes were then selected and used.

Partial purification of receptors

Oocytes (2000–3000) were rinsed and Dounce-homogenized in Hepes buffer [50 mM-Hepes/150 mM-NaCl, pH 7.6, supplemented with phenylmethanesulphonyl fluoride (100 μ M), aprotinin (100 units/ml), bacitracin (1 mM) and Triton X-100 (1%, v/v)]. Liver and skeletal muscle from the posterior legs were removed from Nembutal-anaesthetized frogs, washed in Hepes buffer, and homogenized with a Polytron apparatus. The homogenates were incubated at 4 °C for 30 min with continuous stirring and clarified by centrifugation at 175 000 *g* (*r*_{av} 11.2 cm) for 30 min at 4 °C. The supernatant was subjected to affinity chromatography on WGA-agarose as described in [14]. The eluted material is designated 'partially purified receptors'. The fraction termed 'oocyte lysate' was prepared by adsorption of the high-speed supernatant on a DEAE-Sepharose column, followed by elution in 50 mM-Hepes/250 mM-NaCl, pH 7.6, containing protease inhibitors as above and 0.1% Triton X-100.

Receptor-binding assays

¹²⁵IGF-I (sp. radioactivity 400 μ Ci/ μ g) and ¹²⁵I-insulin (sp. radioactivity 200 μ Ci/ μ g) were prepared by the chloramine-T method. Oocytes (10 or 20 per assay), oocyte lysate (50 μ l/assay) or partially purified receptors (40 μ l/assay) were incubated with the radiolabelled ligand at tracer concentrations (200 pM) for 3 h at 20 °C, in the absence or in the presence of unlabelled peptide, added as indicated. All experimental points were obtained in triplicate. The receptor-bound ¹²⁵I radioactivity was determined by counting as follows. Soluble receptors were precipitated with poly(ethylene glycol) (0.12 g/ml) and bovine immunoglobulins (2 mg/ml). Intact oocytes were washed three times in cold MBS. In both cases, the non-specific background was determined in the presence of 0.1 μ M-insulin or -IGF-I, and subtracted from the total counts. For Scatchard analysis and determination of binding parameters, a computer program was used. This program has been designed in our laboratory and is based on the hypothesis of two classes of binding sites.

Biosynthetic labelling of oocyte receptors

Oocytes (2000–3000) were incubated for 16 h in MBS containing 0.2% BSA, [³⁵S]methionine and [³⁵S]cysteine (1 mCi/ml each), and receptors were partially purified as described above. They were stimulated for 2 h at 20 °C with insulin (1 μ M), phosphorylated in the presence of unlabelled ATP (15 μ M), MgCl₂ (8 mM) and MnCl₂ (4 mM), and immunoprecipitated with

anti-phosphotyrosine antibodies. The precipitate was washed three times in 30 mM-Hepes/30 mM-NaCl, pH 7.6, containing 0.1% Triton X-100, and analysed by SDS/PAGE (7.5%-acrylamide gels) under reducing conditions.

Receptor characterization

Receptor phosphorylation in the presence of [γ -³²P]ATP, analysis by SDS/PAGE and immunoprecipitation with anti-phosphotyrosine or anti-receptor antibodies, were carried out as described elsewhere [15–17]. Antibody anti-C1 was raised against a synthetic peptide corresponding to residues 1309–1326 in the C-terminal region of the insulin receptor β -subunit; this sequence is conserved in both human insulin and IGF-I receptors. This antibody immunoprecipitates 10–40% of the phosphorylated human insulin receptor [17]. Antibody anti-K1 is directed against a peptide corresponding to insulin receptor residues 1247–1261, which are not conserved in the IGF-I receptor (insulin receptor residues are numbered in accordance with Ullrich *et al.* [18]).

Tyrosine kinase activity of oocyte receptors

Partially purified receptors (80 μ l) were incubated with dilutions of either insulin or IGF-I for 60 min at 15 °C. The synthetic substrate poly[Glu-Tyr] was then added (final concn. 0.2 mg/ml). The phosphorylation reaction was initiated by adding 30 μ M- [γ -³²P]ATP (2.5 Ci/mmol), 4 mM-MnCl₂ and 8 mM-MgCl₂, and stopped after 30 min at 20 °C with 100 mM-NaF/20 mM-EDTA. The incorporation of radioactive phosphate into the substrate was determined by a filter-paper assay as described in [19].

RESULTS

Binding of insulin and IGF-I to partially purified oocyte receptors

Specific binding of both insulin and IGF-I was found in partially purified receptors from oocytes isolated by manual dissection. Fig. 1 shows Scatchard analysis of binding data for insulin and IGF-I. The plots were curvilinear, as usually seen with insulin or IGF-I, with a relatively high level of low-affinity binding. Dissociation constants (*K*_d) for the high-affinity binding were calculated from two independent experiments, and were 2.5 $\times 10^{-10}$ M for insulin and 8 $\times 10^{-10}$ M for IGF-I. The estimated number of high-affinity binding sites was, per mg of protein, 1.5 $\times 10^{11}$ for insulin and 22.5 $\times 10^{11}$ for IGF-I. Considering that

Fig. 1. Specific binding of insulin and IGF-I to partially purified oocyte receptors

For each experimental point, 40 μ l of solubilized receptors, partially purified by affinity chromatography on WGA-agarose, was incubated for 2 h at 18–20 °C in triplicate with tracer concentrations (200 pM) of ¹²⁵I-insulin or ¹²⁵I-IGF-I, in the presence of unlabelled insulin ($\square$) or IGF-I ($\blacksquare$) at the indicated concentrations. Binding was determined by scintillation counting of radioactivity of receptors precipitated by poly(ethylene glycol) and bovine immunoglobulins, as described in the Experimental section. Data were analysed by Scatchard plots.

Table 1. Specific binding of insulin and IGF-I to receptors from *Xenopus laevis* oocytes

Intact oocytes (10 per assay) were manually dissected from ovarian lobes or defolliculated by treatment for 2 h at 20 °C with collagenase IA or collagenase II and STI. Solubilized receptors were obtained by recycling an oocyte extract on DEAE-Sepharose [oocyte lysate (50 μ l/assay) corresponding to 10 oocytes] or by chromatography on WGA-agarose [glycoprotein-rich fraction (40 μ l/assay) corresponding to 10 oocytes], as described in the Experimental section. Ligands were used at tracer concentrations (200 pM). Results of a typical experiment are shown and are means of triplicate experimental points; dash indicates below detection limits.

	Insulin bound (fmol/10 oocytes)	IGF-I bound (fmol/10 oocytes)
Intact oocytes		
Manual dissection	0.5	0.6
Collagenase IA	—	—
Collagenase II + STI	0.2	0.7
Solubilized receptors		
Oocyte lysate	0.1	0.8
Glycoprotein-rich fraction	0.2	1.0

the yield of receptor purification on WGA-agarose was 100 % (see Table 1), these data represent (per oocyte) about 7×10^7 insulin-binding sites and 12×10^8 IGF-I-binding sites.

Biosynthetic labelling of oocyte receptors

About 2000 oocytes were incubated during 12 h in the presence of [35 S]methionine and [35 S]cysteine. The glycoprotein-rich fraction of these oocytes was then prepared by affinity chromatography

on WGA-agarose. These partially purified receptors were exposed to insulin (1 μ M), phosphorylated *in vitro* in the presence of unlabelled ATP, and immunoprecipitated by anti-phosphotyrosine antibodies. The concentration of insulin used ensures that both insulin and IGF-I receptors could be observed. An autoradiogram of the precipitate is shown in Fig. 2. 35 S-labelled bands corresponding to receptor α - and β -subunits were detected at 130 kDa for α -subunits, and at 94–105 kDa for β -subunits.

Phosphorylation of partially purified oocyte receptors

Partially purified oocyte receptors were exposed to insulin or IGF-I and then to [γ - 32 P]ATP. After immunoprecipitation with antibodies to phosphotyrosine, proteins were analysed on SDS/PAGE (Fig. 3). Under reducing conditions, two phosphoproteins were revealed at 94 and 105 kDa (lanes 1–5). At 0.1 μ M, insulin and IGF-I stimulated the phosphorylation of both proteins (lanes 2 and 4), but at 1 nM, IGF-I was more potent than insulin (lanes 3 and 5). With 1 nM-insulin (lane 3), the phosphorylation level of the 94 kDa protein was slightly higher than that of the 105 kDa protein, but was clearly below that induced by IGF-I at 1 nM (cf. lane 5). Under non-reducing conditions (right-hand panel), a single phosphoprotein was observed at approx. 350 kDa, in agreement with the expected mobility of heterotetrameric receptors.

Both insulin and IGF-I stimulated the substrate-phosphorylation activity of oocyte receptors in a concentration-dependent manner (Fig. 4). The half-maximally effective concentration (EC_{50}) was 20–50 nM for insulin, and 0.5–1 nM for IGF-I. These data indicate that receptors from oocytes correspond to IGF-I receptors rather than to insulin receptors, in keeping with our binding data showing that partially purified oocyte receptors contain 15 times more IGF-I receptors than insulin receptors.

Fig. 2. Metabolic labelling of oocyte receptors

Intact oocytes (2000–3000) were incubated in [35 S]methionine and [35 S]cysteine (1 mCi/ml each) for 12 h at 20 °C, and the receptors were partially purified by affinity chromatography on WGA-agarose. The eluate was incubated with 1 μ M-insulin, phosphorylated in the presence of unlabelled ATP and precipitated by antibodies to phosphotyrosine (final concn. 5 μ g/ml). The immunoprecipitate was analysed by SDS/PAGE (7.5 % gel) under reducing conditions and autoradiographed: OR, origin.

Fig. 3. Insulin- and IGF-I-induced phosphorylation of partially purified receptors from oocytes

Partially purified receptors were incubated with insulin or IGF-I for 60 min and phosphorylated with [γ - 32 P]ATP for an additional 30 min at 20 °C. The reaction was stopped with NaF/EDTA (100 mM/20 mM) and the samples were exposed to affinity-purified anti-phosphotyrosine Ig (final concn. of 5 μ g/ml). Precipitation was achieved by adding Protein A coupled to Sepharose CL4B. The samples were analysed by SDS/PAGE (5 % gel) under reducing (lanes 1–5) or non-reducing (lanes 6–10) conditions, followed by autoradiography. Abbreviations: OR, origin; INS, insulin.

Fig. 4. Phosphorylation of poly[Glu-Tyr] by partially purified oocyte receptors

Samples of partially purified receptors (80 μ l, in triplicate) were incubated with the indicated insulin ($\square$) or IGF-I ($\blacksquare$) dilutions for 60 min at 20 $^{\circ}$ C. Poly[Glu-Tyr] was then added (final concn. 0.2 mg/ml), and samples were incubated with [γ - 32 P]ATP for 30 min at 20 $^{\circ}$ C. The increase in poly[Glu-Tyr] phosphorylation is shown as a percentage above basal, i.e. in the absence of insulin and IGF-I. The results are expressed as means $\pm$ S.E.M. of three experiments done in triplicate.

Role of oocyte envelopes

Oocytes isolated by manual dissection have conserved most of their envelopes, including follicular cells. These envelopes can be removed by collagenase treatment. Intact oocytes bound insulin as well as IGF-I (Table 1). When oocytes were treated with collagenase IA, which is known to remove all oocyte envelopes [9], binding of both insulin and IGF-I was abolished, suggesting that the enzymic treatment alters the receptors at the oocyte surface. A gentle enzymic stripping, by collagenase II and trypsin inhibitor, removed most, if not all, follicular cells, as judged from observation of oocyte sections under the microscope (results not shown). Under these conditions, the IGF-I binding is unaltered, whereas insulin binding is decreased by 60%. This shows that IGF-I receptors are mostly, if not exclusively, present on the oocyte surface, whereas at least half of the insulin receptors are in oocyte envelopes. Alternatively, this selective loss of insulin binding could be due to a greater sensitivity of insulin receptors than of IGF-I receptors to enzymic degradation.

In solubilized receptor preparations, insulin binding was 5–10 times less than IGF-I binding. Binding was similar in the glycoprotein-rich fraction and in a crude extract, suggesting that the yield of receptor purification on WGA-agarose is near 100%. However, compared with intact oocytes, insulin binding was decreased by a factor of 2–5, whereas IGF-I binding was increased 1.5-fold. This suggests that oocytes may contain a large amount of cryptic or intracellular receptors, a likely explanation since the volume of one oocyte corresponds on average to 10^5 somatic cells. It is also possible that receptor solubilization decreases insulin binding to its receptor, and/or increases IGF-I binding.

Comparison between receptors from oocyte, liver and muscle

To investigate further the nature of the two receptor β -subunits found in oocytes, we have compared oocyte receptors with those isolated from liver and skeletal muscle. Although low IGF-I binding activity was found in liver, these tissues contained mostly insulin receptors (Table 2). This was confirmed by study by SDS/PAGE of the insulin and IGF-I dose-dependent receptor β -subunit phosphorylation pattern (results not shown). The phosphorylation of oocyte, liver and muscle receptors at 1 μ M-

Table 2. Specific binding of insulin and IGF-I to receptors partially purified from *Xenopus laevis* tissues

Receptors were partially purified on WGA-agarose as described in the Experimental section. Ligands were used at tracer concentration (200 pM). Results are means of triplicate points. Protein was measured by the Coomassie Blue method.

	Insulin bound (fmol/mg of protein)	IGF-I bound (fmol/mg of protein)
Oocytes	24	150
Muscle	354	< 10
Liver	1095	40

insulin is shown in Fig. 5. As in the oocyte, two distinct phosphoproteins that shared the characteristics of receptor β -subunits were found at 98 kDa and 92 kDa in liver, and 94 kDa and 85 kDa in muscle. Photoaffinity labelling of liver and muscle receptors with [125 I]-[B2-(2-nitro-4-azidophenylacetyl)]-[B1-Phe]-insulin, performed as described previously [20], revealed receptor α -subunits of 125 kDa in muscle and of 140 kDa in liver (results not shown). In agreement with these differences in molecular mass of α - and β -subunits, muscle receptors migrated slightly faster than liver receptors in non-reducing conditions (Fig. 5, right-hand panel). These data suggest that liver and muscle contain insulin receptors with two distinct β -subunit subtypes.

Fig. 5. Phosphorylation of partially purified receptors from oocytes, liver and muscle

Partially purified receptors (80 μ l for oocytes, 50 μ l for liver and 40 μ l for muscle) were incubated with 1 μ M-insulin for 60 min and phosphorylated with [γ - 32 P]ATP for an additional 30 min at 20 $^{\circ}$ C. The reaction was stopped with NaF/EDTA (100 mM/20 mM) and the samples were exposed to affinity-purified anti-phosphotyrosine Ig (final concn. 5 μ g/ml). Precipitation was achieved by adding Protein A coupled to Sepharose CL4B. The samples were analysed by SDS/PAGE on 7.5% (non-reduced) or 5% (reduced) gels, followed by autoradiography; OR, origin.

Fig. 6. Immunoprecipitation of the phosphorylated receptors

The partially purified receptors from oocytes, liver and muscle, phosphorylated as described in legend to Fig. 5, were immunoprecipitated with affinity-purified anti-phosphotyrosine Ig (PY; final concn. of 5 μ g/ml) or with two anti-peptide antibodies, anti-C1 (final concn. 150 μ g/ml) or anti-K1 (final concn. 90 μ g/ml), directed to, respectively, residues 1309–1326 and 1247–1261 of human insulin receptor. The immunoprecipitates were analysed by SDS/PAGE on 7.5% (reduced) or 5% (non-reduced) gels, followed by autoradiography; OR, origin.

Immunoprecipitation of receptors from oocyte, liver and muscle

The phosphorylated receptors were immunoprecipitated with various antibodies specific for insulin or IGF-I receptors (Fig. 6). In addition to antibodies to phosphotyrosine, which precipitate almost 100% of the phosphorylated receptors, only two antibodies precipitated *Xenopus* receptors, anti-K1 (insulin-receptor-specific), and anti-C1 (directed to a sequence common to insulin and IGF-I receptors). However, even at high antibody concentration, the amount of receptor precipitated was low, suggesting that the avidity of these antibodies for the receptors is weak. In agreement with the expected receptor specificity, anti-C1 precipitated one species from oocyte, muscle and liver receptors, whereas anti-K1 recognized the two insulin-receptor β -subunit subtypes in liver and muscle, but not in oocytes. The molecular species detected by anti-C1 in liver and in muscle was different from that recognized in oocytes, as shown by the failure of anti-K1 to immunoprecipitate oocyte receptors. Moreover, the lower-molecular-mass species of both muscle and liver receptors was precipitated by anti-K1, but not by anti-C1, suggesting immunological differences between the β -subunit subtypes. Treatment of receptors with dithiothreitol before immunoprecipitation, under conditions which cleave the disulphide links between α -subunits and between β -subunits [21], did not modify the immunoprecipitation by either anti-K1 or anti-C1 (results not shown).

We interpret these data to mean that oocytes contain two IGF-I receptors that can be distinguished by their β -subunit subtypes. One of these subtypes, the 94 kDa form, bears the C1 epitope. In liver and muscle, two distinct insulin receptors are also observed. One of these forms contains a β -subunit that bears both C1 and K1 epitopes, whereas the other has a β -subunit of higher electrophoretic mobility that bears the K1 epitope, but not the C1 epitope.

DISCUSSION

The glycoprotein-rich fraction of *Xenopus laevis* oocytes,

obtained by manual dissection, contained high-affinity binding sites for insulin and for IGF-I. Scatchard plots for insulin and IGF-I binding were curvilinear. This curvilinearity is the result of the existence of more than one class of binding sites, or of the presence of a single class of sites with negative co-operativity in binding, or of both [22]. Dissociation constants (K_d) for the high-affinity component of binding (8×10^{-10} M for IGF-I and 2.5×10^{-10} M for insulin) were in agreement with those found in cells that have both insulin and IGF-I receptors, such as IM-9 cells [23]. However, in oocytes, there were about 15 times more high-affinity binding sites for IGF-I than for insulin (per oocyte, 7×10^7 for insulin, and 12×10^8 for IGF-I). This is similar to the quantity of insulin receptors reported by Maller & Koontz [11], who found 4×10^7 high-affinity insulin-binding sites per oocyte isolated by manual dissection. For comparison, in the pre-adipocyte form of 3T3-L1 cells there are 7×10^3 high-affinity insulin-binding sites per cell, whereas 250×10^3 sites are found in the adipocyte form [24]. Assuming that the surface of an oocyte is about 10000-fold greater than that of an adipocyte, the density of insulin receptors in oocytes would be comparable with that of insulin receptors in 3T3-L1 pre-adipocytes, whereas the density of IGF-I receptors is of the same order of magnitude as that of insulin receptors in 3T3-L1 adipocytes. However, these estimates do not take in account the presence of numerous microvilli at both oocyte and adipocyte surfaces.

Oocyte receptors were composed of α -subunits of 130 kDa and β -subunits appearing as a doublet of 94 and 105 kDa in SDS/PAGE. The ligand-induced autophosphorylation pattern of these β -subunits suggested that they represent genuine IGF-I receptor β -subunits [1,7,8]. Moreover, IGF-I was more potent than insulin in activating the oocyte receptor tyrosine kinase (EC_{50} 20–50 nM for insulin and 0.5–1 nM for IGF-I). In agreement with the prevalence of its receptors, IGF-I is 50–100 times more potent than insulin in inducing biological responses [11–13]. We have found that both glucose uptake and oocyte maturation were equally stimulated by high insulin concentrations ($EC_{50} \sim 10$ –50 nM) than by low IGF-I concentrations ($EC_{50} \sim 0.2$ –1 nM) [25]. As for the tyrosine kinase activity of partially purified receptors, these values are in keeping with the K_d of the high-affinity IGF-I binding.

Binding experiments on intact and collagenase-treated oocytes suggest that most of the IGF-I receptors are at the oocyte surface, whereas at least half of the insulin receptors are in the oocyte envelopes. Evidence implicating the insulin receptor kinase in mediation of biological effects in oocytes have been gathered in part from micro-injection studies showing that a monoclonal antibody to the kinase domain of human insulin receptor was able to block oocyte maturation [26] and glucose uptake [12] stimulated by high insulin concentrations. In agreement with Janicot & Lane [12], our results suggest that this antibody effect is likely to be due to the recognition of IGF-I receptors by an antibody directed to an epitope conserved in both insulin and IGF-I receptors.

Oocyte receptors were found to contain two distinct β -subunit subtypes. Similarly, insulin receptors from muscle and liver also contained two β -subunits. Immunoprecipitation of the phosphorylated receptors with anti-peptides to regions of the β -subunits allowed discrimination between the various receptor forms. Anti-C1, which is directed to a C-terminal sequence conserved in both insulin and IGF-I receptors, precipitated one single species in oocytes, liver and muscle. On the contrary, anti-K1 recognized two species in liver and muscle, but not in oocyte, as expected from the specificity of this antibody, which is directed to a sequence unique for the insulin-receptor kinase domain. The β -subunits of oocyte receptors were thus immunologically distinct from those of liver and muscle. Other antibodies used, including

α IR3 (human IGF-I receptor-specific; [27]), B6 (human insulin receptor-specific, [28]) and B2 and B7 (from patients with auto-antibodies to insulin receptor; [29]) failed to precipitate any receptor form. Taken together, our data suggest that the high- and low-molecular-mass β -subunits differ by their C-terminal region. An alternative explanation would be that the smaller β -subunit is a proteolytic derivative of the larger one, in which the distal epitope C1, but not the proximal one K1, has been eliminated by C-terminal truncation. In our view this is unlikely, because anti-C1 recognized the smaller, but not the larger, receptor β -subunit subtype in oocytes. The observation that anti-C1 precipitated only one receptor subtype in oocytes, muscle and liver suggests that the two β -subunits found in each tissue are not associated in the same oligomeric structure as seen in many cell types that express both insulin and IGF-I receptors [21, 30]. However, we cannot exclude that immunoprecipitation by anti-C1 necessitates intramolecular cross-linking of identical β -subunits, thus selecting the fraction of the receptor population with identical β -subunits. In our experiments, insulin receptors of liver and muscle differed from each other only by the electrophoretic mobilities of α - and β -subunits. Therefore, we cannot exclude that muscle and liver contain identical insulin receptor molecules with tissue-specific glycosylations.

To summarize, we have shown that oocytes bear two distinct IGF-I receptor subtypes and low amounts of insulin receptors, whereas in liver and muscle two distinct forms of insulin receptors are present. To date, the possible physiological significance of these multiple receptor forms remains speculative. In the fetal rat, a novel form of IGF-I receptor with a β -subunit of 105 kDa was recently found in skeletal muscle. The expression of this receptor is developmentally regulated and disappears in adult tissue [31]. Two immunologically distinct IGF-I receptors were also observed during central-nervous-system development [32]. These data suggest the existence of a fetal version of IGF-I receptor, a hypothesis that could also explain the presence of an altered IGF-I receptor in the human leukaemic cell line HL-60 [33]. It would thus be interesting to study the fate of the two forms of oocyte IGF-I receptors in fertilized eggs and in embryos. On the other hand, it should be kept in mind that frogs of the genus *Xenopus* are tetraploid animals, as a result of a genome duplication that occurred about 30 million years ago [34]. Some of the duplicated genes may have diverged, as recently shown by the existence of two non-allelic genes that encode distinct insulins in *Xenopus laevis* pancreas [35,36]. So far one *Xenopus* insulin species has been tested, and found to be approx. 1.5–2-fold more potent than pig insulin in binding to human insulin receptors, and in stimulating glucose oxidation in rat adipocytes [36]. Similarly, this genome duplication could account for the presence of multiple subtypes of both insulin and IGF-I receptors. Further studies are needed to determine if these receptor subtypes are specialized in biologically different functions.

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