Insertion of isolated insulin receptors into placental membrane vesicles

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Purified human insulin receptors were inserted into placental plasma-membrane vesicles by fusion of membranes with receptor-lysophosphatidylcholine micelles. Scatchard analysis of insulin binding showed that about 10-15% of the added receptors became inserted into the membrane. The receptor number could be increased about 3-fold, corresponding to approx. 3 pmol of receptor/mg of membrane protein. The receptors became firmly bound to the membrane, as they could not be removed by extensive wash. The insertion of exogenous receptors could be demonstrated by immunoblotting. The inserted insulin receptor had the same insulin-binding affinity as the isolated receptor and the endogenous receptor of the membrane. Insulin binding in the presence or absence of Triton X-100 revealed that more than 80% of the exogenous receptors had a right-side-out orientation. Function of the inserted receptors, as observed by insulin-stimulated autophosphorylation, could be demonstrated. About 80% of the added lysophospholipid, corresponding to approx. 160 nmol of lysophospholipid/mg of membrane protein, became integrated into the membrane and was partly metabolized to phospholipid and to non-esterified fatty acid. The method of insertion of isolated insulin receptors using the natural detergent, lysophospholipid, may be a method for insertion of receptors into intact cells, where the lysophospholipid, as in the plasma-membrane vesicles, will be acylated to phospholipid.

INTRODUCTION

The human placental insulin receptor is an integral membrane protein composed of two α-subunits (Mr 130,000) and two β-subunits (Mr 95,000) disulphide-linked into an α2β2 heterotetrameric complex [1-3]. Cloning of the insulin receptor and determination of the amino acid sequence deduced from the nucleotide sequence of cDNA has revealed information which support earlier biochemical data about the structure of the insulin receptor [4,5]. Recently we have published a model for the quaternary structure of the receptor deduced from electron microscopy of negatively stained preparations of the detergent-solubilized receptor [6].

The molecular mechanism for transmission of the insulin signal over the cell membrane in normal and diseased states is poorly understood. By insertion of isolated native or mutated insulin receptors into cell plasma membranes, information about structure-function relationship of the insulin receptor can be obtained. In this paper, we present data which show that isolated insulin receptors can be inserted into placental membrane vesicles by fusion with receptor-lysophospholipid micelles. Insertion of insulin receptors into membrane vesicles by this method can serve as a model for insertion of insulin receptors into receptor-deficient cells.

MATERIALS AND METHODS

Materials

Phenylmethanesulphonil fluoride (PMSF), trypsin, leupeptin, aprotinin, pepstatin A, 1,10-phenanthroline and benzamidine hydrochloride were all obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. Triton X-100 ("für elektronische Zahlung von Milchzellen" was from Merck, Darmstadt, Germany. CNBr-activated Sepharose 4B was obtained from Pharmacia. Pig insulin and [mono-125I-Tyr-A1]-human insulin were kindly given by Novo Nordisk, Bagsværd, Denmark. Lysophosphatidylcholine (egg) was from Serdary Research Laboratories, London, Ont., Canada, and [1-14C]palmitoyl-lysophosphatidylcholine, [32P]ATP and 125I-Protein A were from Amersham International, Amersham, Bucks., U.K. The Mₙ markers cytochrome c, ovalbumin, BSA, β-galactosidase and cross-linked phosphorylase b were all from Sigma.

Isolation of placental plasma membranes

Plasma membranes were prepared as described by Fujita-Yamaguchi et al. [7] from normal human placentae, obtained within 1 h of delivery. The homogenization was performed at 4°C in 500 ml of 0.25 m-sucrose containing 50 mm-Tris/HC1, pH 7.4, 10 µg of leupeptin/ml and 0.5 mM-PMSF. The crude membranes were washed once and stored in homogenization buffer at a protein concentration of approx. 30 mg/ml at −80°C until use.

Trypsin-treated membranes were prepared by incubation of 12 mg of protein in 0.5 ml of 0.25 m-sucrose containing 50 mm-Tris/HC1, pH 7.4, with 0.4 mg of trypsin for 2 h at room temperature. The membranes were pelleted by centrifugation and suspended in 0.5 ml of 0.25 m-sucrose/50 mm-Tris/HC1, pH 7.4, containing 80 µg of aprotinin.

Purification of insulin receptors and detergent exchange to lysophospholipid

Insulin receptors were isolated as described by Fujita-Yamaguchi et al. [7] by sequential affinity chromatography on wheat-germ agglutinin (WGA) and insulin coupled to Sepharose 4B. The details of the procedure with the modifications introduced by us were as described previously [6].

The dilute receptor preparation eluted from the insulin-Sepharose column in 0.05% Triton X-100 was applied directly to a small WGA-Sepharose column (0.5 ml) in order to concentrate the receptor preparation and to exchange the detergent Triton...
X-100 for lysophosphatidylcholine. The column was washed with 10 ml of 50 mM-Hepes (pH 7.4)/10 % (v/v) glycerol/0.5 mM-PMSF, containing 0.1 % lysophosphatidylcholine and a trace of [14C]lysophosphatidylcholine at a flow rate of 2 ml/h. The receptor was eluted with washing buffer containing 0.3 mM-N-acetylglucosamine in a volume of approx. 1 ml.

Insertion of insulin receptors into placental membrane vesicles

Insulin-receptor-lysophosphatidylcholine micelles (100 μl; 100–200 nm) receptor in 0.1% (2 mM) lysophosphatidylcholine) in the elution buffer from the WGA column were incubated with placental plasma membranes corresponding to 1 mg of protein in a total volume of 0.175 ml of 0.25 m-sucrose/50 mM-Tris/HCl, pH 7.4, containing 1 mM-PMSF, 50 μg of leupeptin/ml, 2 μg of aprotonin/ml, 50 μg- pepstatin A, 2.5 mM-phenanthroline and 25 mM-benzamidine. If stated, the incubation mixture contained 3.0 mM-ATP, 3.0 mM-MgCl2, and 0.2 mM-CoA. The incubation was performed for 37°C for 10 or 30 min in Beckman Airfuge tubes, and separation of unincorporated receptor was performed by centrifugation at 100000 g for 20 min. The pellet was washed once by suspension in 100 μl of 0.25 m-sucrose/50 mM-Tris/HCl, pH 7.4, containing 0.5 mM-KCl, followed by centrifugation. A second wash of the membranes with 0.5 mM-KCl or with 0.05% Triton X-100 did not remove any of the inserted receptor. Therefore in routine experiments only one wash with 0.5 mM-KCl was performed. The washed pellet was suspended in 0.25 m-sucrose/50 mM-Tris/HCl, pH 7.4, containing 0.2 mM-PMSF and 10 μg of leupeptin/ml. The amount of lysophospholipid in the pellet was determined after solubilization in 0.5% Triton X-100 by radioactivity counting.

Scatchard analysis of insulin binding was performed either on the suspended membranes or after solubilization in Triton X-100. Insertion of receptors into membrane vesicles was always compared with control experiments performed under the same conditions as described above, except that membranes were incubated with the WGA elution buffer containing 0.1% lysophosphatidylcholine without receptor.

Insulin-binding assay

Insulin binding to isolated insulin receptors, placental membranes or Triton-X-100-solubilized membranes was performed by incubation of 15 μl of receptors with 0.1 nm-[125I]insulin for 16 h at 4°C in a final volume of 0.1 ml of 50 mM-Tris/HCl (pH 7.4)/0.1 % BSA/0.1 mM-PMSF. Free [125I]insulin was separated from the bound hormone by addition of 25 μl of 0.4 % bovine γ-globulin and 125 μl of 20 % poly(ethylene glycol) (M6000). After 20 min at 4°C samples were centrifuged at 12000 g for 4 min in a Beckman Microfuge. The pellet, suspended in 300 μl of 0.5% Triton X-100, and the supernatant were both counted for radioactivity. Non-specific binding was determined in the presence of 4 μM unlabelled insulin. For Scatchard analysis a concentration range of 0.4–32 nm unlabelled insulin was used.

Production of antisera

Antiserum against purified insulin receptors were prepared by immunization of rabbits by subcutaneous injection of 50 μg of antigen into the back. The immunization scheme of Harboe & Ingild [8] was followed, Freund's complete adjuvant being used in the first injection and the incomplete adjuvant in the following injections. Either the antiserum was used as such, or the immunoglobulin fraction was isolated by chromatography on Protein A-Sepharose.

Autophosphorylation

Control vesicles or vesicles enriched with insulin receptors were produced as described above by incubation of placental membrane vesicles with either lysophospholipid or insulin-receptor-lysophospholipid micelles but without addition of Mg-ATP, MnCl2 and CoA. Autophosphorylation was performed on the two samples by using either intact vesicles or a Triton-X-100-solubilized preparation. The four samples were phosphorylated by incubation with 1 μM-insulin for 30 min at room temperature, followed by addition of 8 mM-MgCl2, 2 mM-MnCl2 and [γ-32P]ATP (20 μCi) at a final ATP concentration of 50 μM. Phosphorylation was stopped after 15 min by addition of ATP, EDTA and sodium orthovanadate to final concentrations of 3 mM, 15 mM and 1 mM respectively. The two samples, where autophosphorylation was performed on intact vesicles, were pelleted by centrifugation, and Triton-X-100-solubilized preparations were made. Portions of all four samples were analysed directly by SDS/PAGE, and portions of the two samples corresponding to autophosphorylated Triton-X-100-solubilized material were immunoprecipitated before analysis on SDS/PAGE. For immunoprecipitation the samples were incubated with the IgG fraction of the anti-insulin-receptor antiserum (50 μg of protein), Protein A-Sepharose (25 μl) and 50 mM-dithiothreitol for 2 h at 4°C. The beads were washed three times with 10 mM-Tris/HCl, pH 7.4, containing 150 mM-NaCl, 1% Triton X-100, 0.5% sodium deoxycholate and 0.1 % SDS and solubilized by boiling in 250 mM-Tris/HCl, pH 6.8, containing 8 M-urea, 5 % SDS and 100 mM-dithiothreitol. All samples were analysed by SDS/PAGE under reducing conditions, stained with silver or Coomassie Blue and analysed by autoradiography on Kodak XAR 5 film with Cronex Lightning Plus intensifying screens. The intensity of the bands was quantified with a LKB Ultrascan XL laser densitometer.

SDS/PAGE and Western blotting

SDS/PAGE was performed as described by Laemmli [9] in 6% acrylamide gels (non-reducing conditions) or 13% acrylamide gels (reducing conditions). Western blotting was performed as described by Erickson et al. [10]. The proteins were transferred to nitrocellulose paper by using a transfer buffer of 25 mM-Tris/HCl 192 mM-glycine, pH 8.3, containing 0.1 % SDS and 20 % (v/v) methanol. The primary antibody was used in a 1:50 dilution. The blots were washed and developed either with a polyclonal swine anti-rabbit immunoglobulin coupled to peroxidase (Dako, Glostrup, Denmark) in a 1:400 dilution, with colour development performed according to the manufacturer's instruction, or with [125I]-Protein A (2.5 μCi/μg; 200000 d.p.m./ml). In the last method, the protein bands were detected by autoradiography and quantified by densitometry as described above.

Lipid extraction and analysis

Lipid extraction of the washed pellet was performed by adding chloroform/methanol (2:1, v/v) as described by Fox & Zilver- smit [11]. The extracted lipid was dissolved in a known volume of chloroform/methanol/water (25:15:2, by vol.). Analysis of the conversion of lysophosphatidylcholine was performed by t.l.c. in the solvent system chloroform/methanol/water (65:25:4, by vol.). The spots were made visible by iodine vapour, and after evaporation the spots were scraped from the plate into scintillation vials and the radioactivity was counted in 4 ml of UltimaGold (Packard Instruments).

RESULTS

Incorporation and metabolism of lysophosphatidylcholine

Lysophosphatidylcholine was incorporated into membranes to a high degree. In all experiments a 75–85% incorporation of
Table 1. Metabolism of lysophosphatidylcholine incorporated into placental plasma membranes

The incubation mixture contained 1 mg of protein and 200 nmol of lysophosphatidylcholine in a total volume of 0.175 ml. The Mg-ATP and CoA concentrations were 3.0 mM and 0.2 mM respectively. The incubation temperature was 37 °C. The values represent the average of duplicate determinations. Abbreviations: PC, phosphatidylcholine; NEFA, non-esterified fatty acids.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Time (min)</th>
<th>¹⁴C incorporation (nmol)</th>
<th>Lyso-PC (nmol)</th>
<th>PC (nmol)</th>
<th>NEFA (nmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>160</td>
<td>132</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>160</td>
<td>102</td>
<td>11</td>
<td>47</td>
</tr>
<tr>
<td>+ Mg-ATP, CoA</td>
<td>30</td>
<td>160</td>
<td>91</td>
<td>45</td>
<td>24</td>
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</tbody>
</table>

Table 2. Insertion of insulin receptors into placental plasma membranes

The incubation mixture contained 1 mg of protein either as native membranes or as membranes treated with trypsin, 18 pmol of insulin receptors and 200 nmol of lysophosphatidylcholine (lyso-PC) in a total volume of 0.175 ml. The Mg-ATP, MnCl₂, and CoA concentrations were 3.0, 0.4 and 0.2 mM respectively. The incubation time was 30 min at 37 °C. Insulin binding was performed on intact membrane vesicles as described in the Materials and methods section.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Insulin receptors (pmol)</th>
<th>Increase in receptors (pmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membranes</td>
<td>1.7</td>
<td>_</td>
</tr>
<tr>
<td>Membranes + lyso-PC</td>
<td>1.7</td>
<td>_</td>
</tr>
<tr>
<td>+ receptors</td>
<td>3.5</td>
<td>1.8</td>
</tr>
<tr>
<td>+ receptors + Mg-ATP, CoA</td>
<td>5.2</td>
<td>3.5</td>
</tr>
<tr>
<td>+ receptors + Mg-ATP, Mn²⁺, CoA</td>
<td>5.8</td>
<td>4.1</td>
</tr>
<tr>
<td>Trypsin-treated membranes</td>
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<td>_</td>
</tr>
<tr>
<td>+ receptor</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>+ receptor + Mg-ATP, CoA</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>+ receptor + Mg-ATP, Mn²⁺, CoA</td>
<td>2.3</td>
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</tr>
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</table>

Insulin binding was performed on intact placental membrane vesicles before (□) and after (○) incubation with 14 pmol of insulin receptors and 200 nmol of lysophosphatidylcholine, in buffer containing 3.0 mM-Mg-ATP and 0.2 mM-CoA for 30 min at 37 °C. The total volume was 0.175 ml. The ratio of bound to free insulin was plotted against the amount of insulin bound to the membranes.

Fig. 1. Scatchard analysis of insulin binding

Insulin binding was performed on intact placental membrane vesicles before (□) and after (○) incubation with 14 pmol of insulin receptors and 200 nmol of lysophosphatidylcholine, in buffer containing 3.0 mM-Mg-ATP and 0.2 mM-CoA for 30 min at 37 °C. The total volume was 0.175 ml. The ratio of bound to free insulin was plotted against the amount of insulin bound to the membranes.

Insolation of insulin receptors

Measured as an increase in insulin binding, isolated insulin receptors as protein–lysophosphatidylcholine micelles were inserted into placental membranes, as shown in Table 2. The addition of lysophosphatidylcholine micelles without receptors to placental membrane vesicles did not affect insulin binding of the endogenous receptors, but in all subsequent experiments the increase in number of receptors was always compared with control vesicles to which lysophospholipid without receptors was added. The addition of the insulin receptor was, like the incorporation of the lysophospholipid, not dependent on time of incubation. The amount of endogenous insulin receptors in 1 mg of membrane protein could be doubled when incubated with 100 µl of a 180 mM receptor preparation in 0.1% (2 mM) of lysophosphatidylcholine solution in a total volume of 0.175 ml at 37 °C for 10–30 min. The number of receptors could be further increased by incubation in the presence of Mg-ATP and CoA, an incubation system which enhances the acylation of lysophosphatidylcholine to phosphatidylcholine. On addition of MnCl₂ to the incubation system containing Mg-ATP and CoA, no significant further increase in insertion of receptors into membrane vesicles was seen. Scatchard analysis showed an increase in number of receptors with a Kᵣ of 0.5–1 nm, similar to the Kᵣ of the endogenous receptors (Fig. 1), and to the Kᵣ of the isolated insulin receptor [6].

Trypsin-treated membranes in which endogenous receptors had been destroyed could also be used for insertion of insulin receptors. The amount of receptors inserted into trypsin-treated membranes was lower than the amount inserted into native membranes (Table 2). However, the same effect of increased insertion of receptors with addition of Mg-ATP, CoA and MnCl₂ was observed.

The amount of receptors inserted was dependent on the amount of vesicles in the incubation mixture. A linear relationship
Fig. 2. Effect of increasing amount of protein or exogenously added insulin receptors on receptor insertion into placental plasma membranes

(a) An increasing amount of placental plasma-membrane protein was incubated with 14 pmol of insulin receptors and 200 nmol of lysophosphatidylcholine, in buffer containing 3.0 mm-Mg-ATP and 0.2 mm-CoA in a total volume of 0.175 ml at 37 °C for 30 min. (b) Placental plasma-membrane protein (1 mg) was incubated with increasing amount of insulin receptors and 2 mmol of lysophosphatidylcholine in buffer containing 3.0 mm-Mg-ATP and 0.2 mm-CoA in a total volume of 0.175 ml at 37 °C for 10 min. In both experiments the insertion of insulin receptors was determined by insulin binding.

Fig. 3. Immunoblotting of insulin receptors

Placental plasma membranes were incubated with lysophosphatidylcholine or receptor-lysophosphatidylcholine (30 pmol of receptor) in an incubation system containing 3.0 mm-Mg-ATP and 0.2 mm-CoA for 30 min at 37 °C as described in the Materials and methods section. SDS/PAGE was performed in a 13 %-acrylamide gel under reducing conditions. The proteins were transferred to nitrocellulose paper and incubated overnight in a 1:50 dilution of anti-insulin receptor antisemur. After washing, the blots were developed either (a) with horseradish-peroxidase-conjugated secondary antibody or (b) by autoradiography after incubation with 125I-Protein A. Lane 1, placental membranes (approx. 250 μg of protein); lane 2, placental membranes enriched in receptors (approx. 250 μg of protein); lane 3, pure insulin receptors (approx. 0.50 μg). Positions of α and β subunits of the receptor are marked.

Fig. 4. Autophosphorylation of insulin receptors in placental membranes and placental membranes enriched in insulin receptors

Placental membranes incubated with lysophosphatidylcholine (lanes 1 and 2) or with insulin receptor (IR)-lysophosphatidylcholine (lanes 3 and 4) were autophosphorylated either as intact vesicles (lanes 1 and 3) or after addition of Triton X-100 (TX; lanes 2 and 4) as described in the Materials and methods section. Approx. 125 μg of placental protein was analysed by reducing SDS/PAGE and autoradiography. In lane 5 is shown the immunoprecipitate of the sample identical with that in lane 2, and in lane 6 is shown the immunoprecipitate of the sample identical with that in lane 4. M₀ (× 10⁶) markers are shown at left.

of receptors inserted into 0.5–2.5 mg of protein was obtained (Fig. 2). With a fixed amount of protein, the number of receptors inserted was within the concentration range studied, dependent in a linear fashion on the amount of receptor in the incubation mixture (Fig. 2).

The concentration of the receptor preparation is limited, as it is not possible to isolate large amounts of pure, fully functional, receptor. At the same time, only a limited amount of lysophosphatidylcholine can be added to membranes without their solubilization. However, by incubation of 1 mg of placental membrane protein with 60 pmol of receptor as lysophosphatidylcholine micelles, the receptor number was only increased 4.4-fold indicating that a saturation point for exogenous incorporated receptors could be obtained.

Insertion of insulin receptors was measured by insulin binding either directly to membranes or to membranes solubilized in Triton X-100. The increase in number of receptors, measured directly on the membranes, was 80–100 % of the values obtained if measured on a Triton-X-100-solubilized preparation, indicating that most receptors had a right-side-out orientation. The inserted insulin receptors became firmly bound to the membrane. Thus they could not be removed either by extensive washing with high salt (0.5 m-KCl) or with low concentrations of detergent (0.05 % Triton X-100).

Insertion of insulin receptors could be detected by immunoblotting after SDS/PAGE. Fig. 3(a) shows an immunoblot with peroxidase-conjugated immunoglobulin as secondary antibody, and Fig. 3(b) shows an autoradiogram of an immunoblot using 125I-Protein A to detect the antigen. From both experiments, it is evident that our antibody recognizes the β subunit in preference to the α subunit of the receptor. Densitometric scanning of the autoradiogram revealed a 3.5-times higher blackening of the film on the positions of the β subunit of the receptor-enriched
samples (lane 2) compared with the control (lane 1). This value is comparable with the insulin-binding data of the same experiment where the binding increased from 1.8 (1.4) to 6.3 (5.9) pmol per mg of protein. Values in parentheses are those obtained on a Triton-X-100-solubilized preparation of the membranes.

Insulin-sensitive autophosphorylation was performed on vesicles enriched with exogenously added receptors, by using either intact vesicles or vesicles which were made leaky by addition of Triton X-100. The results show (Fig. 4) that no phosphorylation could be demonstrated without Triton X-100. Only after addition of Triton X-100 could the receptor kinase be monitored. Autoradiography of the gel showed several labelled bands, one with an Mr corresponding to the β subunit of the insulin receptor together with bands of lower Mr. The phosphorylated bands of lower Mr are unknown. Phosphorylated proteins of Mr 120000, 60000 and 45000 have been seen in other tissues [12]. They might also be degradation products of the β subunit of the insulin receptor, but in that case they are not precipitated with our antibody. Immunoprecipitation revealed only one band corresponding to the β subunit of the insulin receptor, and the enrichment of the placentale membrane vesicles with insulin receptors is evident. Densitometric scanning of the film revealed a 1.8-times higher blackening in the receptor-enriched samples compared with the control. This increase in receptors is comparable with the insulin-binding data in Table 2, where a doubling of receptors is obtained when Mg-ATP, MnCl2 and CoA are omitted for insertion. Control vesicles and vesicles enriched in receptors without added Triton X-100 both lacked insulin-stimulated autophosphorylation, indicating that the vesicles were tightly sealed and the receptors, endogenous as well as exogenous, most probably had the same orientation.

**DISCUSSION**

In previous papers we have shown that isolated membrane proteins such as aminopeptidase from intestinal microvillus membranes [13] and cytochrome b5 from liver endoplasmic reticulum [14,15] can be incorporated into preformed liposomes or into membrane vesicles by fusion with protein–lysophosphatidylcholine micelles. In the present paper we show that a complex membrane protein like the insulin receptor, which in contrast with the above-mentioned proteins contains both an extracellular and an intracellular domain, can also be incorporated into membranes by the same procedure.

Addition of insulin-receptor–lysophosphatidylcholine micelles to placentale membrane vesicles led to an increase in number of receptors, as measured by insulin binding. The inserted receptors became firmly bound to the membrane; thus they could not be removed by extensive washing. The increase in insulin binding of the enriched vesicles monitored on intact vesicles was 80–100% of the increase measured on a Triton-X-100-solubilized preparation of the vesicles, indicating that most of the isolated receptors had a right-side-out orientation. Close to a 100% right-side-out orientation has been obtained by the present method used for insertion of other proteins into lipid vesicles or membranes [13–15]. Considering the model for the structure of the insulin receptor [6], it is most likely that the receptor should be inserted into membrane vesicles via the less voluminous β subunit of the molecule.

The $K_0$ of 0.5–1 nm of the insulin receptors after fusion was the same as the $K_0$ of the receptors in the native membranes and the $K_0$ of the isolated receptor [6]. Thus no alteration in the affinity of the receptors for insulin after insertion was observed. The exogenously added insulin receptors also showed the function of insulin-stimulated autophosphorylation. Similarly to the endogenous receptors, no phosphorylation could be demonstrated in intact vesicles, confirming the data on insulin binding that most of the receptors had a right-side-out orientation.

The lysophosphatidylcholine incorporated along with the receptor was partly converted into phosphatidylcholine or degraded to non-esterified fatty acid. The conversion into phosphatidylcholine was greatly enhanced by increasing incubation time and by adding Mg-ATP and CoA to the incubation mixture, in order to convert non-esterified fatty acids into acyl-CoA, the substrate for acyltransferases.

The amount of receptors inserted into membranes was enhanced by Mg-ATP and CoA. In this mixture the receptor will become autophosphorylated. It has been reported that autophosphorylation alters the conformation of the β subunit of the insulin receptor [16,17]. It is not known whether this conformational change can explain the increased insertion of the receptor into the lipid bilayer.

Fusion of insulin-receptor-containing phospholipid vesicles with a cell line (Madin–Darby canine kidney) by using 20% poly(ethylene glycol) [18] has led to insertion of insulin receptors into receptor-deficient cells. The amount of receptors inserted into 10⁶ cells is in the order of 5 fmol. The method described in the present paper could also be a method for insertion of insulin receptors into receptor-deficient cells. In our method 10–15% of the receptors become inserted into the membrane vesicles, leading to an increase in the amount of receptors of approx. 4 pmol/mg of protein. As 1 mg of plasma-membrane protein is roughly comparable with 50 × 10⁶ cells, the present method can insert about 15 times more receptors than by the method of Hofmann et al. [18]. Preliminary experiments with insertion of insulin receptors as protein–lysophospholipid micelles into isolated rat liver hepatocytes indicate that the plasma membrane of intact cells can incorporate lysophospholipid in amounts comparable with those used in this study, the cells still being viable. Therefore the present method may increase the level of receptors in cells to the same value as obtained in transfected cells (10⁴–10⁶ receptors per cell). Furthermore, the present method also has the advantage over the method of Hofmann et al. [18] that no reconstitution of insulin receptors into phospholipid vesicles is needed. In the present method only a simple exchange of Triton X-100 for lysophospholipid has to be performed before fusion can take place. The present method makes use of the natural detergent, lysophospholipid, which is a component present, although in small amounts, in all biological membranes, and the lysophospholipid incorporated together with the receptor will be converted into the natural membrane component, phospholipid, by the acyltransferases present in the plasma membrane.

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**REFERENCES**

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