Somatomedin receptor of human placenta: Solubilization, photolabeling, partial purification, and comparison with insulin receptor

(isoceptors/insulin-like growth factors/growth hormone)

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ABSTRACT Using a recently isolated human basic somatomedin (basic SM) similar to insulin-like growth factor I (IGF-I), we studied both the photoaffinity-labeled and unlabeled basic-SM receptor solubilized from human placental cell membranes. Unlike the result with the insulin receptor, high yields of soluble basic-SM-binding activity are obtained with Triton X-100. The soluble basic-SM receptor retains high affinity (K_a ≈ 0.3 nM) peptide-specific binding of basic SM, similar to the binding present in particulate placenta membranes; the receptor exhibits a comparatively low affinity for insulin (K_d ≈ 3 µM). On Sepharose 6B, like the crude soluble insulin receptor, the basic-SM receptor migrates as a species with an apparent Stokes radius of 7.2 nm; unlike the insulin receptor, the basic-SM receptor does not, under similar conditions, yield a smaller binding species (apparent Stokes radius 3.6 nm). Upon photoaffinity labeling with [125I]-labeled basic SM, one principal specifically labeled constituent is detected. Upon gel electrophoresis in the presence of 2-mercaptoethanol, the photolabeled constituent, like the insulin receptor, migrates as a species with an apparent molecular weight of about 140,000; in the absence of reducing agent, a molecular weight greater than 240,000 is observed. Lectin-agarose affinity chromatography yields a 30-fold purification both of the basic-SM-binding activity and the photolabeled constituent. Anti-insulin receptor antibody does not appear to precipitate the basic-SM receptor. We conclude that the basic-SM receptor of human placenta is a glycoprotein, remarkably similar to (an isoceptor) but distinct from the insulin receptor previously characterized in this tissue.

The somatomedins (SM) are a group of growth hormone-dependent, serum-borne, polypeptide growth factors that have in common the ability to stimulate proteoglycan synthesis in cartilage and to mimic the actions of insulin in a variety of extraskelatal tissues. The somatomedins account for the insulin-like activity in human serum that is not neutralized by anti-insulin antibody (so-called nonsuppressible insulin-like activity or NSILA; recently renamed insulin-like growth factors or IGFs). Recent evidence suggests that all of the reported somatomedins may fall into two main groups based on their isoelectric points. The basic group (pls greater than 7.4) includes insulin-like growth factor-I (IGF-I) (1), SM-C (2), and a basic SM that has been purified in our laboratory (3). The acidic-neural group (pl less than 7.4) includes IGF-II (1), SM-A (4), and multiplication-stimulating activity (5). The basic group of somatomedins are highly similar in terms of their molecular weights, pls, NH2-terminal amino acid sequences (1-3), and immunoreactivity (3, 6, 7). IGF-I (similar to our basic SM) and IGF-II have different NH2-terminal amino acid sequences but share a sequence homology with each other and with proinsulin (8). Thus, there is a complex relationship between insulin, the somatomedins, and the peptide-specific receptors for each of these polypeptides. Although distinct receptors for the somatomedins have been detected in fat cells (9), fibroblasts (9, 10), and placenta (11), the sequence homologies lead to cross-specificity of binding at the receptor sites, so that insulin at high concentrations (micromolar) can occupy the SM receptors and vice versa.

In view of the cross-specificity of the ligand recognition properties of the receptors for insulin and the SMs, we have become interested in comparing the physicochemical properties of the insulin receptor with those of the receptors for the SMs. In the present work, we report the solubilization, photoaffinity labeling, characterization, and partial purification of the human placenta receptor for human basic SM. Our results indicate a high degree of physicochemical similarity between the basic-SM receptor and the receptor for insulin.

MATERIALS AND METHODS

Iodination of Basic SM and Insulin. Highly purified basic SM was isolated as reported (3). This basic SM had a potency of 4000 units/mg by hypophysectomized rat cartilage bioassay. Insulin was a gift from Eli Lilly. Peptides were iodinated by a modification of the chloramine-T method (6) to specific activities of 100-150 µCi of basic SM and 80-100 µCi of insulin per µg (1 Ci = 3.7 x 10⁶ becquerels).

Preparation of Membranes and Membrane Extract. A crude "microsomal" membrane fraction from term human placenta cells was prepared from the placental basal plate by homogenization and differential centrifugation, essentially as described (12, 13). Membranes were solubilized (6 mg of membrane protein per ml of detergent solution) for 30 min at 24°C with 0.5% Triton X-100 or 0.15-0.5% Ammonyx-LO (Onyx Chemical, Hoboken, NJ) in 50 mM Tris-HCl (pH 7.4). The extract was clarified by centrifugation at 150,000 x g for 70 min at 4°C. Before use, Ammonyx-LO-solubilized membrane preparations were made 0.5% in Triton X-100 and were dialyzed for 17 hr at 4°C against 0.5% Triton X-100 in 50 mM Tris-HCl (pH 7.4).

Binding Assay. Solubilized membrane protein (≈50 µg of protein in 0.1 ml) was incubated with radiolabeled basic SM (10,000-100,000 cpm) or insulin (≈100,000 cpm) with or without unlabeled basic-SM or insulin (≈1 µg of unlabeled peptide per ml) in Tris-HCl buffer (pH 7.4) containing 5 mg of bovine serum albumin per ml (final vol, 0.3 ml). Samples in 12 x 75 mm poly styrene tubes were equilibrated overnight at 4°C.

Abbreviations: IGF, insulin-like growth factor; SM, somatomedin.

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for basic SM and for 60 min at 24°C for the measurement of insulin binding. Specific ligand binding was measured by the polyethylene glycol method as described (14).

**Photoaffinity Labeling.** Basic SM or insulin was first iodinated by using a modification of the procedure described by Spivak et al. (15). Glass tubes (12 × 75 mm) were freshly coated with water-insoluble oxidizing agent 1,3,4,6-tetrachloro-3-α,6-diphenylglycrol (IODO-GEN; Pierce). Basic SM (10 μg) or insulin (5 μg) in 200 μl of 0.1 M sodium phosphate (pH 7.5) was added to the IODO-GEN tube. Carrier-free 125I (1.5–3 μCi, Amersham) was then added (=5 μl), and the solution was vortexed vigorously for 40 sec. The reaction mixture was filtered through a plug of glass wool in a Pasteur pipette and made up to 2 ml with the sodium phosphate buffer. A photoaffinity labeling derivative was then prepared by the addition of 30 μl of N-succinimidyl 6-[(4′-azido-2′-nitrophenylamino)hexanolate (Pierce; 1 mg/ml in dimethyl sulfoxide) as described for epidermal growth factor (16) and was used without further purification. The derivative of either basic SM or insulin (200–400 ng/0.1 ml) was incubated with membranes (4 mg of protein) in the dark at 4°C (total vol. 4 ml) either with or without an excess (≈1 μg/ml) of undervitamized peptide. After 45 min, the azide group was activated by illumination (two 200-W incandescent bulbs at 10 cm) at 4°C for 20 min. Photolabeled membrane was collected, washed by centrifugation (48,000 × g for 20 min), and solubilized in 0.5–1 ml of buffer containing 1% detergent (either Triton X-100 or Ammonyx-LO). The photolabeled receptor was then analyzed by NaDodSO4/polyacrylamide gel electrophoresis (7.5% gels; ref. 16) either before or after partial purification on lectin-agarose columns. Labeled proteins were visualized by autoradiography as described (17).

**Immunoprecipitation of Receptor.** Rabbit antiserum from animals immunized with purified rat liver insulin receptor (Lot A-410) was provided by S. Jacobs (Burroughs Wellcome, Research Triangle Park, NC). Soluble receptor (100 μl in detergent-containing buffer) was first equilibrated overnight at 4°C in a total volume of 300 μl with radiolabeled ligand (either insulin or basic SM) either in the presence or absence of an excess of the appropriate unlabeled peptide. Anti-receptor antibody (10 μl) was then added and the antibody–receptor complex was allowed to form for 6 hr at 4°C. Second antibody (goat anti-rabbit immunoglobulin; Calbiochem) was then added (200 μl of reconstituted goat antibody; capable of reacting with 8–10 μl of rabbit serum), and the immunoprecipitate was allowed to form for 48–72 hr at 4°C. Control experiments were performed with nonimmune rabbit serum. Radioactivity in the washed immunoprecipitate was measured by crystal scintillation counting.

**RESULTS**

In keeping with previous observations (18), extraction of placenta membranes with Triton X-100 yielded relatively small amounts of soluble insulin-binding activity, compared to the insulin-binding activity present in the membranes prior to solubilization; this result suggests a lability of the insulin receptor under the conditions of extraction (Table 1). In contrast, more than 60% of the basic-SM-binding activity was recovered in soluble form from placenta membranes by using Triton X-100 (Table 1); the detergent Ammonyx-LO was equally effective in solubilizing basic-SM-binding activity but, as detailed previously (18), yielded a greater amount of soluble insulin-binding activity than did Triton X-100 (data not shown). Thus, there appears to be a differential solubilization of the receptors for insulin and basic SM, depending on the detergent used. Most studies were done with Triton X-100-solubilized basic-SM receptor.

![Graph](image)

**Fig. 1.** Binding of 125I-labeled basic SM to receptor from placenta membrane. (A) Binding isotherms were determined for particulate membrane preparation (C) (100 μg of membrane protein per assay tube) and solubilized membrane preparation (G) (50 μg of protein per assay tube). Separation of bound from free peptide was achieved by centrifugation (2000 × g for 30 min) for particulate membrane and by the polyethylene glycol method for solubilized membrane. (B) Binding competition of unlabelled insulin and basic SM for the binding of 125I-labeled basic SM by solubilized receptor was measured by the polyethylene glycol method. Binding is expressed as the fraction (B/B0) of radiolabeled basic SM bound in the presence (B) and absence (B0) of competing unlabelled ligand. c, SM; e, insulin. Kd = 0.20 nM (SM) and 2.80 μM (insulin).

**Table 1.** Solubilization of basic-SM- and insulin-binding activities by Triton X-100

<table>
<thead>
<tr>
<th>Sample</th>
<th>Basic-SM binding, %</th>
<th>Insulin binding, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant</td>
<td>64 ± 4</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Pellet</td>
<td>50 ± 2</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>Net recovery of binding activity</td>
<td>114 ± 6</td>
<td>56 ± 3</td>
</tr>
</tbody>
</table>

Membranes (≈6–10 mg) were solubilized for 30 min at 24°C in 11 ml of 0.5% Triton X-100 in 50 mM Tris-HCl (pH 7.4). The extract was clarified by centrifugation (150,000 × g for 70 min at 4°C), and the pellet was washed twice with detergent-free buffer. The washed pellet was resuspended in an equal volume of buffer, and aliquots of the soluble extract and washed membrane suspension were assayed for ligand binding; the detergent concentration in the binding assay was ≤0.04%.

The values represent the average of two independent estimates (±1/2 range) of the percentage of binding present in the supernatant and pellet after detergent extraction, relative to the binding present in the original membrane suspension.

The binding of 125I-labeled basic SM by solubilized receptor paralleled binding by the particulate receptor; binding was saturable and of high affinity (Kd = 100 pM for the radiolabeled peptide; Fig. 1A). From the binding-competition curves (Fig. 1B), the inhibition constant (K) for the binding of unlabeled...
By using developed methods (16), it was possible to photolabel both the insulin receptor and the basic-SM receptor in placenta membranes. Upon electrophoretic analysis and autoradiography, the major soluble constituent photolabeled with the 125I-labeled basic-SM photoprobe exhibited a mobility (molecular weight ≈ 140,000) very close to that of the photolabeled insulin receptor (Fig. 3). Photolabeling of the basic-SM receptor was virtually abolished in the presence of an excess of unlabeled basic SM, but not in the presence of unlabeled insulin (Fig. 3, channels a–d). In the absence of 2-mercaptoethanol, the photolabeled basic-SM receptor, like the insulin receptor (21, 22), migrated upon electrophoresis as a constituent with a molecular weight greater than 240,000 (Fig. 3, channel e). As noted above, both photolabeled receptors could be adsorbed to and eluted from columns of concanavalin A-agarose (Fig. 3, channels f–i).

The results of preliminary studies with anti-insulin receptor antibody are shown in Table 2. Studies using soluble placenta membrane preparations, containing primarily basic-SM-binding activity and little insulin-binding activity (a Triton X-100 extract), revealed that little or no basic-SM-binding activity was precipitated with the anti-receptor antibody. In contrast, the anti-receptor antibody was capable of precipitating substantial amounts of insulin receptor either from solubilized rat liver protein from photoaffinity-labeled placenta membrane. Channels: a–d, analysis of protein that was photolabeled with 125I-labeled basic SM in the absence of an excess unlabeled basic SM (channels a and d), in the presence of unlabeled basic SM (channel b), and in the presence of unlabeled insulin (channel c); e, receptor photolabeled with 125I-labeled basic SM and subjected to electrophoretic analysis in the absence of 2-mercaptoethanol; f and g, analysis of receptor that was first photolabeled with 125I-labeled basic SM and then solubilized for chromatographic analysis by using columns of concanavalin A-agarose (300 μl) as described (16), showing the electrophoretic-autoradiographic analysis of the unadsorbed fraction (channel f) and the adsorbed material that was eluted from the column with 0.2 M α-d-methyl-mannopyranoside (channel g); h and i, analysis of insulin receptor that was first photolabeled with 125I-labeled insulin and then solubilized for chromatographic analysis with concanavalin A-agarose columns (300 μl), showing the unadsorbed fraction (channel h) and the adsorbed material that was eluted from the column with 0.2 M α-d-methyl-mannopyranoside (channel i). The molecular weight calibration shown ×10^{-5} applies only to the experiment depicted in channels a and b; human erythrocyte membrane marker proteins were used for calibration. Separate electropherograms are depicted by channels a and b, c–e, and f–i. The relative mobility of the photolabeled receptor, determined in separate experiments, did not differ appreciably.

FIG. 3. Electrophoretic analysis and autoradiography of soluble protein from photoaffinity-labeled placenta membrane. Channels: a–d, analysis of protein that was photolabeled with 125I-labeled basic SM in the absence of an excess unlabeled basic SM (channels a and d), in the presence of unlabeled basic SM (channel b), and in the presence of unlabeled insulin (channel c); e, receptor photolabeled with 125I-labeled basic SM and subjected to electrophoretic analysis in the absence of 2-mercaptoethanol; f and g, analysis of receptor that was first photolabeled with 125I-labeled basic SM and then solubilized for chromatographic analysis by using columns of concanavalin A-agarose (300 μl) as described (16), showing the electrophoretic-autoradiographic analysis of the unadsorbed fraction (channel f) and the adsorbed material that was eluted from the column with 0.2 M α-d-methyl-mannopyranoside (channel g); h and i, analysis of insulin receptor that was first photolabeled with 125I-labeled insulin and then solubilized for chromatographic analysis with concanavalin A-agarose columns (300 μl), showing the unadsorbed fraction (channel h) and the adsorbed material that was eluted from the column with 0.2 M α-d-methyl-mannopyranoside (channel i). The molecular weight calibration shown ×10^{-5} applies only to the experiment depicted in channels a and b; human erythrocyte membrane marker proteins were used for calibration. Separate electropherograms are depicted by channels a and b, c–e, and f–i. The relative mobility of the photolabeled receptor, determined in separate experiments, did not differ appreciably.

FIG. 2. Chromatography on Sepharose 6B of solubilized SM-binding material. Aliquots (0.2 ml) of the Triton X-100-soluble extract from plasma membranes (940 μg of protein) were subjected to chromatography on a column (1.5 × 85 cm) of Sepharose 6B equilibrated with phosphate buffer (20) containing 0.1% Triton X-100. (A) Two identical samples were analyzed subsequent to equilibration with 125I-labeled basic SM (40 fmol per assay tube; 0.1 nM) in the absence (c) or presence (e) of unlabeled basic SM (1 nM). (B) A second aliquot was chromatographed first, and the specific binding of 125I-labeled basic SM (15 μM) in the effluent fractions was subsequently measured by the polyethylene glycol method.

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membranes or from soluble human placenta membrane extracts (Ammonyx-LO) containing large amounts of insulin-binding activity (Table 2). In placenta membrane extracts containing comparable amounts of both insulin receptor and basic-SM receptor, the immunoprecipitation results using radiolabeled basic SM were difficult to interpret because of the appreciable binding of basic SM to the insulin receptor. The results with radiolabeled basic SM were further complicated by the presence in nonimmune rabbit serum of a basic-SM non-receptor binding protein that appears to coprecipitate with the immunoprecipitate.

### DISCUSSION

Our studies indicate a striking similarity between the placenta insulin receptor and the receptor for basic SM (similar to IGF-I). Both receptors exhibit a very similar chromatographic behavior (Sepharose 6B) and both photolabeled receptor constituents have virtually the same electrophoretic mobilities in NaDodSO₄-containing polyacrylamide gels (molecular weights ≈ 140,000 in the presence of 2-mercaptoethanol). Further, upon electrophoretic analysis, both receptors behave as high molecular weight species (>240,000) in the absence of reducing agent. The ability of both photolabeled receptors to adsorb to the same lectin-agarose columns also may point to similarities in the oligosaccharide moities of the two receptor species. These overall properties of the insulin and basic-SM receptors are remarkably different from the characteristics of the receptor for epidermal growth factor-urogastrone, which is also present in the soluble placenta membrane extracts (24).

The binding competition data unequivocally distinguish between the soluble binding site for basic SM and the binding site for insulin. However, given the similarities between the soluble placenta components that bind insulin and basic SM, one might reasonably ask if both polypeptides are bound to different regions of the same receptor macromolecule. Thus, it is important to underline the differences between the receptors. First, the recovery data indicate that the two receptors are not solubilized in equal proportions from the membranes. Second, although both soluble receptors show a major binding species (Sepharose 6B) with a Stokes radius of about 7.2 nm, only the insulin receptor, under these conditions, shows a smaller binding component (≈3.8 nm) that has been repeatedly detected in a variety of tissues (18, 20, 25). Finally, it appears that under extraction conditions that yield principally soluble SM-binding activity without insulin-binding activity, no SM-binding activity is precipitated by anti-insulin receptor antibody. Further work will be required to determine unequivocally whether there is some immunological crossreactivity between the receptors for insulin and basic SM. On the whole, our findings are highly suggestive that placental cell membranes contain two distinct but very similar receptor macromolecules (isoreceptors)—one for insulin and another for basic SM. This result is in accord with other data suggesting that the receptors for multiplication-stimulating activity (26) and SM-A (4, 27) are distinct from the receptor for insulin.

We believe that our photolabeling method labels principally the 140,000 molecular weight or α subunit of the insulin-receptor oligomer described by others (21, 22). Our results indicate the presence of a very similar subunit in the basic-SM receptor. Further studies using peptide-mapping methods to determine the structural homology between the subunits of the two receptors may establish the presence or absence of a β subunit in the basic SM receptor, similar to the one present in the insulin receptor (28, ǂ). In addition, further studies comparing the basic-SM receptor with the receptors for the acidic–neutral group of somatomedin (IGF-II, SM-A, and multiplication-stimulating activity) may help to elucidate further the relationship between the various polypeptides with SM-like activities.

We are indebted to Dr. Steven Jacobs for providing the anti-insulin receptor antibody used for our studies. The expert technical assistance of J. Dankova is gratefully acknowledged. These studies were made possible by grants from the Medical Research Council of Canada (R.M.B. and M.D.H.) and from the March of Dimes—Birth Defects Foundation (to M.D.H.).

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Table 2. Immunoprecipitation of insulin- and basic-SM binding activities

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Addition</th>
<th>Specific binding activity, counts per 5 min</th>
<th>Specific 125I-Labeled insulin</th>
<th>Specific 125I-Labeled basic SM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total</td>
<td>Nonspecific</td>
<td>Specific</td>
</tr>
<tr>
<td>1</td>
<td>Antiserum, 10 μl</td>
<td>24,800</td>
<td>1,790</td>
<td>23,010</td>
</tr>
<tr>
<td></td>
<td>Nonimmune serum, 10 μl</td>
<td>3,810</td>
<td>2,430</td>
<td>1,380</td>
</tr>
<tr>
<td>2</td>
<td>Antiserum, 2 μl</td>
<td>28,920</td>
<td>2,810</td>
<td>26,110</td>
</tr>
<tr>
<td></td>
<td>Nonimmune serum, 2 μl</td>
<td>3,530</td>
<td>2,430</td>
<td>1,110</td>
</tr>
<tr>
<td></td>
<td>Polystyrene glycol</td>
<td>103,600</td>
<td>20,700</td>
<td>82,900</td>
</tr>
</tbody>
</table>

Solubilized placenta (experiments 1 and 2) or rat liver membranes (experiment 1, for insulin binding only) were either used directly (experiment 2, placenta membranes only) or after partial purification with wheat germ agglutinin-Sepharose (experiment 1). In experiment 1, the eluted placenta fractions (100 μl) from wheat germ agglutinin-Sepharose contained SM binding but were devoid of insulin binding; thus an analogous fraction from solubilized liver membranes, eluted from wheat germ agglutinin-Sepharose, was used as an insulin-receptor-containing control. In experiment 2, the placenta extract (0.15% Ammonyx-LO) contained comparable amounts of both insulin-binding and basic-SM-binding activity as indicated by the polyethylene glycol assay. This extract (100 μl) was used directly for the immunoprecipitation experiment. In experiment 1, extracts were equilibrated with 156,000 cpm of 125I-labeled insulin and 87,800 cpm of 125I-labeled basic SM; in experiment 2 samples were equilibrated with 122,000 cpm of 125I-labeled insulin and 132,000 cpm of 125I-labeled basic SM. Immunoprecipitates were counted for 5 min to minimize counting error; values represent the means of duplicate or triplicate determinations that routinely agreed within 10%. In control experiments, it can be demonstrated that no specific radioligand binding is observed in the absence of membrane extract (ref. 25; unpublished data).
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