Three monoclonal antibodies, designated αIR-1, αIR-2, and αIR-3, were prepared by fusing FO myeloma cells with spleen cells from a mouse immunized with a partially purified preparation of insulin receptors from human placenta. These antibodies were characterized by their ability to immunoprecipitate solubilized receptors labeled with 125I-insulin or 125I-somatmedin-C in the presence or absence of various concentrations of unlabeled insulin or somatomedin-C. αIR-1 preferentially immunoprecipitates insulin receptors and also less effectively immunoprecipitates somatomedin-C receptors, while αIR-2 and αIR-3 preferentially immunoprecipitate somatomedin-C receptors, but may also weakly immunoprecipitate insulin receptors.

These three monoclonal antibodies, as well as A410, a rabbit polyclonal antibody, were used to immunoprecipitate insulin and somatomedin-C receptors from solubilized human lymphoid (IM-9) cells and human placenta membranes that had been 125I-labeled with lactoperoxidase. Analysis of the immunoprecipitates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicates that both receptors are composed of α and β subunits. The β subunit of the insulin receptor (immunoprecipitated by αIR-1 and A410) has a slightly more rapid mobility than the corresponding subunit of the somatomedin-C receptor (immunoprecipitated by αIR-2 and αIR-3). Interestingly, the α subunit of the placenta somatomedin-C receptor has a slightly faster mobility than its counterpart from IM-9 cells.

Immunoprecipitation of receptor that had been reduced and denatured to generate isolated subunits indicates that αIR-2 and αIR-3 interact with the α subunit of the somatomedin-C receptor while A410 interacts with both subunits of the insulin receptor. αIR-1 failed to react with reduced and denatured receptors.

Materials and Methods

Receptor Purification—Human placenta membranes were solubilized with 5% Triton X-100, and insulin receptor was purified by sequential chromatography on concanavalin A-Sepharose, insulin-Sepharose, and wheat germ agglutinin-Sepharose columns. About 5-10 ng of receptor protein was obtained per placenta.

Production of Monoclonal Antibodies—Three SJL mice (Jackson Laboratories, Bar Harbor, ME) were infected subcutaneously, with wheat germ agglutinin-Sepharose eluate containing 3 μg of receptor, emulsified in an equal volume of complete Freund's adjuvant, and boosted three times at 3 week intervals with a similar amount of purified receptor emulsified in incomplete Freund's adjuvant. All mice developed antiserum that immunoprecipitated receptors labeled with 125I-insulin and 125I-somatostatin (see Table I for details of assay). The mouse with the highest titer of antibodies to insulin receptors received an i.v. boost of 10 μg of receptor. Three days later, it was sacrificed and its lymph node and spleen cells were fused with FO myeloma cells (Cell Distribution Center, Salk Institute, La Jolla, CA) to achieve an initial cloning stage; hybrids were seeded at a low density with 5-10 ng of receptor protein per well. A patient with insulin resistance and acanthosis nigricans was injected with about 135,000 and 90,000 (3, 4, 6-12). These are thought to form disulfide-linked heterotetramers containing two copies of each type of subunit (3, 4, 6-8, 10-12). Antibodies from a patient with insulin resistance and acanthosis nigricans have been shown to inhibit the binding of both insulin and somatomedin-C to their respective receptors, suggesting that the receptors are also immunochemically similar (13). Somatomedin-C also binds with relatively high affinity to insulin-like growth factor II receptors. This receptor is structurally different from somatomedin-C and insulin receptors, and has little or no affinity for insulin (3, 4, 14).

The present studies describe three monoclonal antibodies to insulin and somatomedin-C receptors. These are used to investigate the immunochemical cross-reactivity of the two receptors and to identify their subunits in human placenta and IM-9 cells. Some properties of αIR-1 have been described previously (15).
density using peritoneal exudate cells from SJL mice as feeder (21). Hybridomas grew to numbers exceeding 10000 cells/cm² of culture medium in 26 out of 1000 wells. Supernatants were screened for antibodies that immunoprecipitated insulin or somatomedin-C receptors as described in the legend to Table 1. Six wells were initially positive for antibodies to insulin receptors. These and only these were also positive for antibodies to somatomedin-C receptors. Lines from three of these wells eventually died out or stopped producing antibody. Hybridomas from the remaining three wells were serially subcloned by limiting dilution four times. The resulting clones and the antibodies they produce have been designated aIR-1, aIR-2, and aIR-3. Antibodies to insulin were harvested from ascites fluid of Balb/C X SJL hybrids (Jackson Laboratories) inoculated with these cell lines. aIR-1 and aIR-3 were further purified on DEAE-cellulose equilibrated with 10 mM potassium phosphate, pH 8.0. aIR-2 was retained on DEAE-cellulose under these conditions, and ascites fluid was used directly without further purification.

All three antibodies are IgG1 (determined by Mono AB-ID EIA Kit, Zymed Laboratories, Burlingham, CA).

Iodination of Cells and Membranes—IM-9 cells were labeled with 125I by using lactoperoxidase (22). The labeled cells were washed with phosphate-buffered saline, solubilized by vortexing with 1% Triton X-100 and 0.1% bovine serum albumin and 0.1% Tween 20, and centrifuged at 3,000 × g to sediment membranes. Supernatants were used for antibodies to somatomedin-C receptors which are not precipitated by this protocol.]

The column was washed with 20 ml of the Triton-containing buffer, and the labeled glycoproteins were eluted with 0.5 M NaC1, pH 7.7, containing 0.1% bovine serum albumin and 0.1% Triton X-100. In one set of control tubes, 100 µg of lactoperoxidase was added followed by 2 µCi of [125I]NaI. A 20-µl aliquot of 10⁻³ M H₂O₂ was added every 4 min for 12 min. The membranes were then washed three times by centrifugation at 50,000 × g for 30 min with 8 ml of phosphate-buffered saline. The membrane pellet was solubilized with 2% Triton X-100 in 50 mM Tris-HCl, pH 7.7, containing 1 mg/ml of bacitracin and 20 µg/ml of phenylmethylsulfonyl fluoride. After 30 min, the solubilized placenta membranes were centrifuged at 100,000 × g for 1 h. The supernatant was diluted with three volumes of Tris-HCl, pH 7.7, containing 1 mM CaCl₂ and 1 mM MgCl₂, and 0.5% N-acetylglucosamine in 50 mM Tris-HCl containing 0.2% Triton X-100, 1 mg/ml of bacitracin, and 20 µg/ml of phenylmethylsulfonyl fluoride.

RESULTS

Table 1 illustrates the ability of the three monoclonal antibodies to immunoprecipitate receptor bound 125I-insulin and 125I-somatostatin-C. aIR-1 immunoprecipitates considerably more bound 125I-insulin and 125I-somatostatin-C than does normal mouse serum. If solubilized placenta is omitted from the assay (or if it is heated to 70 °C for 10 min (data not shown)), there is no specific immunoprecipitation of either labeled hormone by aIR-1. This indicates that the antibody labeled with 125I-somatostatin-C receptor-bound hormone (or in the case of somatomedin-C, a binding protein in serum or ascites fluid), but with hormone binding proteins present in placenta membranes. The ability of insulin to inhibit the immunoprecipitation of the labeled hormones indicates that these binding proteins are saturable and have a relatively high affinity for insulin. aIR-2 and aIR-3 both immunoprecipitate more receptor-bound 125I-insulin than normal serum but considerably less than aIR-1. Both antibodies immunoprecipitate similar amounts of bound 125I-somatostatin-C. As with aIR-1, specific immunoprecipitation of both labeled hormones by aIR-2 and aIR-3 is dependent on the presence of solubilized placenta and is inhibited by native insulin, or by heat treating the solubilized placenta (data not shown).

Receptor Specificity—Since in these studies, 125I-insulin and 125I-somatostatin-C are immunoprecipitated as labeled hormone-receptor complexes, the potency of unlabeled hormones to compete for receptor binding, and thereby inhibit immunoprecipitation of labeled hormone, reflects their specificity for the receptor. This can be used to identify the receptor to which the labeled hormone is bound when it is immunoprecipitated.

The concentrations of unlabeled insulin and somatomedin-C that inhibit the immunoprecipitation of 125I-insulin by aIR-1 (Fig. 1A) are similar to those previously reported to inhibit the binding of 125I-insulin to the insulin receptor (2, 4). This suggests that the 125I-insulin that is immunoprecipitated by aIR-1 (Fig. 1A) is bound mainly to the insulin receptor, and that aIR-1, therefore, recognizes insulin receptors. Similarly, the concentrations of unlabeled insulin and somatomedin-C that inhibit the immunoprecipitation of 125I-somatostatin-C by aIR-2 and aIR-3 (Fig. 1, E and F) are similar to those previously reported to inhibit the binding of 125I-somatostatin-C to the somatomedin-C receptor (2-5). This suggests that aIR-2 and aIR-3 recognize the somatomedin-C receptor.

The competition binding curves in Fig. 1, B, C, and D are more complex. Since 125I-insulin will bind weakly to the somatomedin C receptor and since aIR-2 and aIR-3 immunoprecipitate the somatomedin-C receptor, it is possible that the relatively small amounts of 125I-insulin immunoprecipitated by these antibodies are bound entirely to somatomedin-C receptors. However, the data (Fig. 1, B and C) are not consistent with this interpretation. The potency of native insulin to inhibit the immunoprecipitation of 125I-insulin by aIR-2 and aIR-3 is too high, and the potency of unlabeled somatomedin-C is too low (Fig. 1, B and C) for all the immunoprecipitated 125I-insulin to be bound to the somatomedin-C receptor. Similarly, the potency of unlabeled insulin is too low and the potency of unlabeled somatomedin-C is too high for the 125I-insulin immunoprecipitated by these antibodies to be bound entirely to insulin receptors. The simplest explanation for these data is that 125I-insulin immunoprecipitated by aIR-2 and aIR-3 is bound to a combination of insulin receptors and somatomedin-C receptors. The flat slopes of the competition curves (Fig. 1, B and C) are consistent with the presence of more than one type of receptor. This reasoning suggests that aIR-2 and aIR-3 do immunoprecipitate insulin receptors, although at the concentration of antibody used, considerably less effectively than aIR-1. Similarly, the 125I-somatostatin-C immunoprecipitated by aIR-1 (Fig. 1D) appears to be bound to a mixture of insulin and somatomedin-C receptors, suggesting that aIR-1 weakly recognizes
specifically immunoprecipitates a labeled band with a molecular weight of 135,000 (Fig. 2, lane 17). Labeling of this band is readily inhibited by 100 ng/ml of insulin (data not shown). When 125I-insulin is used as the labeled peptide, aIR-2 and aIR-3 immunoprecipitated a 132,000-Mr band that was heavily labeled in the absence of unlabeled peptides (Fig. 2, lanes 1–4), with 100 ng/ml of somatomedin-C (Fig. 2, lanes 5–8), with 100 ng/ml of insulin (Fig. 2, lanes 9–12), or with both 100 ng/ml of somatomedin-C and insulin (Fig. 2, lanes 13–16). 125I-somatmedin-C was then covalently cross-linked to the receptor to which it was bound with disuccinimidyl suberate. The membranes were solubilized with Triton X-100, immunoprecipitated with normal mouse serum, aIR-1, aIR-2 or aIR-3, and analyzed by SDS-polyacrylamide gel electrophoresis.

aIR-2 and aIR-3 immunoprecipitated a 132,000-Mr band that was heavily labeled in the absence of somatomedin-C (Fig. 2, lanes 3 and 4). Labeling of this band was readily inhibited by 100 ng/ml of somatomedin-C (Fig. 2, lanes 7 and 8), but not inhibited by 100 ng/ml of insulin (Fig. 2, lanes 11 and 12). Because of its relative affinity for insulin and somatomedin-C and its electrophoretic mobility, this band appears to be the α subunit of the somatomedin-C receptor.

In the absence of unlabeled peptides, the band immunoprecipitated by aIR-1 (Fig. 2, lane 2) is less heavily labeled than those immunoprecipitated by aIR-2 or aIR-3. In addition, it is broader and has a portion with a slightly slower electrophoretic mobility. Furthermore, its labeling is only partially inhibited by unlabeled somatomedin-C (Fig. 2, lane 6) and is also partially inhibited by unlabeled insulin (Fig. 2, lane 10), suggesting that this band is composed of α subunits of both insulin and somatomedin-C receptors.

When similar studies are carried out using 125I-insulin as the labeled peptide instead of 125I-somatmedin-C, aIR-1 specifically immunoprecipitates a labeled band with a molecular weight of 135,000 (Fig. 2, lane 17). Labeling of this band is readily inhibited by 100 ng/ml of insulin (data not shown). When 125I-insulin is used as the labeled peptide, aIR-2 and aIR-3 were readily inhibited by 100 ng/ml of insulin (data not shown). Because of its relative affinity for insulin and somatomedin-C, this band appears to be the α subunit of the somatomedin-C receptor.

In addition, it was also partially inhibited by unlabeled insulin (Fig. 2, lanes 13–16). 125I-somatmedin-C was then covalently cross-linked to the receptor to which it was bound with disuccinimidyl suberate. The membranes were solubilized with Triton X-100, immunoprecipitated with normal mouse serum, aIR-1, aIR-2 or aIR-3, and analyzed by SDS-polyacrylamide gel electrophoresis.

aIR-2 and aIR-3 immunoprecipitated a 132,000-Mr band that was heavily labeled in the absence of somatomedin-C (Fig. 2, lanes 1–4), with 100 ng/ml of somatomedin-C (Fig. 2, lanes 5–8), with 100 ng/ml of insulin (Fig. 2, lanes 9–12), or with both 100 ng/ml of somatomedin-C and insulin (Fig. 2, lanes 13–16). 125I-somatmedin-C was then covalently cross-linked to the receptor to which it was bound with disuccinimidyl suberate. The membranes were solubilized with Triton X-100, immunoprecipitated with normal mouse serum, aIR-1, aIR-2 or aIR-3, and analyzed by SDS-polyacrylamide gel electrophoresis.

aIR-2 and aIR-3 immunoprecipitated a 132,000-Mr band that was heavily labeled in the absence of somatomedin-C (Fig. 2, lanes 3 and 4). Labeling of this band was readily inhibited by 100 ng/ml of somatomedin-C (Fig. 2, lanes 7 and 8), but not inhibited by 100 ng/ml of insulin (Fig. 2, lanes 11 and 12). Because of its relative affinity for insulin and somatomedin-C and its electrophoretic mobility, this band appears to be the α subunit of the somatomedin-C receptor.

In the absence of unlabeled peptides, the band immunoprecipitated by aIR-1 (Fig. 2, lane 2) is less heavily labeled than those immunoprecipitated by aIR-2 or aIR-3. In addition, it is broader and has a portion with a slightly slower electrophoretic mobility. Furthermore, its labeling is only partially inhibited by unlabeled somatomedin-C (Fig. 2, lane 6) and is also partially inhibited by unlabeled insulin (Fig. 2, lane 10), suggesting that this band is composed of α subunits of both insulin and somatomedin-C receptors.


Fig. 3. Immunoprecipitation of labeled receptor from iodinated human placenta membranes and IM-9 cells. A. 125I-labeled placenta membrane glycoproteins (1.2 \times 10^6 cpm) were incubated in 50 mM Tris-HCl, pH 7.7, containing 0.2% Triton X-100, 0.1% bovine albumin, 1 mg/ml of bacitracin, and 20 µg/ml of phenylmethylsulfonyl fluoride with: lane 1, normal mouse serum diluted 1:300; lane 2, normal mouse serum diluted 1:300 plus aIR-1 (19 µg of IgG/ml); lane 3, normal mouse serum diluted 1:300 plus aIR-2 (ascites fluid 1:420); lane 4, normal mouse serum diluted 1:300 plus aIR-3 (11 µg of IgG/ml); lane 5, 100 µg/ml of preimmune rabbit IgG; lane 6, 85 µg/ml of A410. After 8 h at 4 °C, 20 µl of anti-mouse serum (Cappel) diluted 1:3 was added to the tubes containing mouse immunoglobulin, and 20 µl of fixed staphylococci bearing protein A (Pansorbin) was added to tubes containing rabbit immunoglobulin, and the incubation was continued overnight at 4 °C. The immunoprecipitates were then washed three times with 4 ml of Tris-HCl, containing 0.2% Triton X-100, 0.1% bovine albumin, 1 mg/ml of bacitracin, and 20 µg/ml of phenylmethylsulfonyl fluoride with: lane 2, normal mouse serum diluted 1:300; lane 3, normal mouse serum diluted 1:300 plus aIR-2 (ascites fluid 1:420); lane 4, normal mouse serum diluted 1:300 plus aIR-3 (11 µg of IgG/ml); lane 5, 100 µg/ml of preimmune rabbit IgG; lane 6, A410.

B. 125I-labeled IM-9 cell membrane glycoproteins (3.2 \times 10^6 cpm) were incubated in 50 mM Tris-HCl, pH 7.7, containing 0.2% Triton X-100, 0.1% bovine albumin, 1 mg/ml of bacitracin, and 20 µg/ml of phenylmethylsulfonyl fluoride with: lane 1, normal mouse serum; lane 2, aIR-1; lane 3, aIR-2; lane 4, aIR-3; lane 5, preimmune rabbit IgG; lane 6, A410.

\(aR-3\) fail to produce detectable specific immunoprecipitation of affinity-labeled bands (data not shown). This is consistent with the relatively weak ability of \(aR-2\) or \(aR-3\) to immunoprecipitate receptor labeled with 125I-insulin as is indicated by Table I and Fig. 1.

**Immunoprecipitation of Lactoperoxidase-labeled Receptors**—To further demonstrate that these antibodies interact directly with receptors for insulin and somatomedin-C, and to establish their specificity, we examined their ability to immunoprecipitate 125I-labeled membrane glycoproteins from human placenta and IM-9 cells. As previously described (15), \(aR-1\) specifically immunoprecipitated two polypeptides with apparent molecular weights of 135,000 and 90,000 from both human placenta and IM-9 cells (Fig. 3A, lanes 2 and Fig. 3B, lane 2). Polypeptides with similar molecular weights were immunoprecipitated by A410 (Fig. 3A, lane 6 and Fig. 3B, lane 6), a rabbit antiserum to rat liver insulin receptor (23). These bands correspond to the \(\alpha\) and \(\beta\) subunits of the insulin receptor described previously by several laboratories (6–9, 22).

\(aR-2\) and \(aR-3\) also specifically immunoprecipitated two polypeptides with apparent molecular weights of approximately 135,000 and 90,000 (Fig. 3A, lanes 3 and 4, and Fig. 3B, lanes 3 and 4). Because of the specificity of \(aR-2\) and \(aR-3\), these presumably are subunits of the somatomedin-C receptor. In both placenta and IM-9 cells, the broad band corresponding to the \(\beta\) subunit has a slightly slower mobility (apparent \(M=92,000–98,000\)) than the corresponding subunit of the insulin receptor. In some gels, this band appears as a doublet, the faint lower component having a mobility similar to the corresponding subunit of the insulin receptor. Interestingly, in human placenta, the \(\alpha\) subunit of the somatomedin-C receptor (immunoprecipitated by \(aR-2\) or \(aR-3\)) has a slightly faster mobility (apparent \(M=132,000\)) than the corresponding subunit of the somatomedin-C receptor from IM-9 cells (apparent \(M=136,000\)) or of the insulin receptor (immunoprecipitated by \(aR-1\) or A410) from either tissue (apparent \(M=135,000\)).

In order to determine with which subunit these antibodies interact, immunoprecipitation studies were performed with iodinated placenta membrane glycoproteins that had been treated with dithiothreitol and SDS to dissociate receptor subunits (Fig. 4). After this treatment, neither subunit is immunoprecipitated by \(aR-1\), perhaps indicating that this antibody recognizes an epitope that is destroyed by reduction and denaturation. \(aR-2\) and \(aR-3\) specifically immunoprecipitate the \(\alpha\) subunit of the somatomedin-C receptor. A410 immunoprecipitates both the \(\alpha\) and \(\beta\) subunits of the insulin receptor. Since A410 is polyclonal, this does not necessarily
Antibodies to Receptors for Insulin and Somatomedin-C

**Figure 4. Immunoprecipitation of reduced and denatured receptor.** $^{125}$I-labeled placenta glycoproteins were reduced and denatured by incubation with 1% SDS and 5 mM dithiothreitol for 5 min at room temperature. The dithiothreitol was then quenched with 12 mM N-ethylmaleimide and the denatured proteins diluted 20-fold with 1% albumin in 50 mM Tris-HCl, pH 7.7, containing 0.2% Triton X-100, 1 mg/ml of bacitracin, and 20 µg/ml of phenylmethylsulfonyl fluoride. The reduced and denatured labeled receptor was then immunoprecipitated as described in Fig. 3. Lane 1, normal mouse serum; lane 2, aIR-1; lane 3, aIR-2; lane 4, aIR-3; lane 5, preimmune rabbit serum; lane 6, A410.

**Discussion**

The present studies describe three separate monoclonal antibodies which react predominantly with insulin receptors (aIR-1) or somatomedin-C receptors (aIR-2 and aIR-3). We have interpreted the data in Fig. 1, B C, and D as indicating that immunoprecipitated labeled ligand is bound to a combination of insulin and somatomedin-C receptors, and therefore, that each antibody can react with both receptors. The ability of aIR-1 to immunoprecipitate both insulin and somatomedin-C receptors is also suggested by affinity cross-linking studies (Fig. 2). However, other explanations for the data are also possible. For example, the antibodies may recognize a third type of receptor that is distinct from both insulin and somatomedin-C receptors and that binds both of these ligands with intermediate affinity. The insulin like growth factor II receptor is a possible candidate, but it may be proven to be a receptor for insulin (3, 4), while the receptors responsible for labeled ligand binding in Fig. 1, B C, and D do. Furthermore, polyacrylamide gel electrophoresis of the immunoprecipitates of lactoperoxidase labeled cells and membranes reveals no labeled bands in the 220–260-kDa range (Fig. 3) which could correspond to the insulin-like growth factor II receptor (3, 4, 14).

aIR-2 and aIR-3 have many similar properties. Both are IgG$_1$(x), both have selectivity for somatomedin-C receptors, and both recognize the reduced and denatured 135,000-molecular weight subunit. However, they are clearly different antibodies. aIR-2 has more stringent specificity for somatomedin-C receptors.

**Acknowledgment**—We thank Stella Cook for her excellent technical assistance.

**REFERENCES**

1. Rinderknecht, E., and Humbel, R. E. (1978) *J. Biol. Chem.* 253, 2769–2776
2. Van Wyk, J. J., Svoboda, M. E., and Underwood, L. E. (1980) *J. Clin. Endocrinol. Metab.* 50, 206–208
3. Kasuga, M., Van Obberghen, E., Nisley, S. P., and Rechler, M. M. (1981) *J. Biol. Chem.* 256, 5305–5308
4. Massague, J., and Czech, M. P. (1982) *J. Biol. Chem.* 257, 5038–5045
5. Bhaumick, B., Goren, H. J., and Bala, R. M. (1981) *Horm. Metab. Res.* 13, 515–518
6. Jacobs, S., Hazum, E., and Cuatrecasas, P. (1980) *J. Biol. Chem.* 255, 6937–6940
7. Jacobs, S., and Cuatrecasas, P. (1981) *Endocr. Rev.* 2, 251–263
8. Massaguit, J., Pilch, P. F., and Czech, M. P. (1980) *Proc. Natl. Acad. Sci. U. S. A.* 77, 7137–7141
9. Yip, C. C., Yeung, C. W. T., Moule, M. L. (1980) *Biochemistry* 19, 70–76
10. Bhaumick, B., Bala, R. M., and Hollenberg, M. D. (1981) *Proc. Natl. Acad. Sci. U. S. A.* 78, 4279–4283
11. Chernausek, S. D., Jacobs, S., and Van Wyk, J. J. (1981) *Biochemistry* 20, 7345–7350
12. Kasuga, M., Van Obberghen, E., Nissley, S. P., and Rechler, M. M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 1864–1868
13. Rosenfeld, R. G., Baldwin, D., Jr., Dollar, L. A., Hintz, R. L., Oletsky, J. M., and Rubenstein, A. (1981) Diabetes 30, 976–982
14. Massague, J., Guillette, B. J., and Czech, M. P. (1981) J. Biol. Chem. 256, 2122–2125
15. Kull, F. C., Jr., Jacobs, S., Su, Y.-F., and Cuatrecasas, P. (1982) Biochem. Biophys. Res. Commun. 106, 1019–1026
16. Cuatrecasas, P., and Tell, G. P. E. (1973) Proc. Natl. Acad. Sci. U. S. A. 70, 485–489
17. Jacobs, S., Shechter, Y., Bissel, K., and Cuatrecasas, P. (1977) Biochem. Biophys. Res. Commun. 77, 981–988
18. Siegel, T. W., Ganguly, S., Jacobs, S., Rosen, O. M., and Rubin, C. S. (1981) J. Biol. Chem. 256, 9266–9273
19. Bennett, A., Daly, F. T., and Hintz, R. L. (1981) Diabetes 30, Suppl. 1, 55A
20. Merril, C. R., Goldman, D., Sedman, S. A., and Ebert, M. (1980) Science 211, 1437–1438
21. Fazekas de St. Groth, S., and Scheidegger, D. (1980) J. Immunol. Methods 35, 1–21
22. Kasuga, M., Kahn, C. R., Hedo, J. A., Van Obberghen, E., and Yamada, K. M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 6917–6921
23. Jacobs, S., Chang, K.-J., and Cuatrecasas, P. (1978) Science 200, 1283–1284
24. Massague, J., Pilch, P. F., and Czech, M. P. (1981) J. Biol. Chem. 256, 3182–3190
25. Yip, C. C., Moule, M. L., and Yeung, C. W. T. (1982) Biochemistry 21, 2940–2945
26. Kasuga, M., Hedo, J. A., Yamada, K. M., and Kahn, C. R. (1982) J. Biol. Chem. 257, 10392–10399
27. Klapper, D. C., Svoboda, M. E., and Van Wyk, J. J. (1983) Endocrinology, in press