We have investigated the effect of glucose on insulin-like growth factor II (IGF-II) binding to, and intracellular phosphorylation of, the IGF-II/mannose 6-phosphate (M6P) receptor in the insulin-secreting cell line RINm5F. Glucose, at a concentration of 3 mM, significantly increased binding of IGF-II to the cells. A further increase of the binding was observed at a glucose concentration of 10 mM. Scatchard analysis showed that the increased binding was caused by an increased number of the receptors rather than changes in affinity. This effect of glucose was also demonstrated in another insulin-secreting cell line HIT as well as in the human erythroleukemia cell line K562. Affinity cross-linking of the receptors rather than changes in affinity. This effect of glucose on IGF-II binding was mimicked by fructose (10 mM), but not by 3-O-methylglucose (10 mM), and was abolished by the protein kinase C (PKC) inhibitor calphostin C, or down-regulation of PKC, but not by the protein phosphatase inhibitor, okadaic acid. Glucose dose dependently stimulated phosphorylation of the IGF-II/M6P receptor, an effect that was inhibited by down-regulation of PKC activity. This study suggests that the distribution of the IGF-II/M6P receptor in insulin-secreting cells can be regulated by glucose-induced phosphorylation, a mechanism mediated by PKC.

The insulin-like growth factor II (IGF-II)/mannose 6-phosphate (M6P) receptor, distinct from the insulin-like growth factor I (IGF-I) receptor, the insulin receptor, and the recently identified IGF-II-specific receptor (1), consists of a single polypeptide chain with a large extracellular domain, containing binding sites for ligands, and a short cytosolic region, which lacks intrinsic protein kinase activities (2, 3). The IGF-II binding site is localized to residues 1508–1566 of the extracellular domain of the receptor (4). A sequence in the cytoplasmic domain has been proposed to be a potential substrate for protein kinase C (PKC) or cyclic-AMP-dependent protein kinase (2). In addition, potential tyrosine, serine, and threonine phosphorylation sites have been identified (3, 5).

Both IGF-II and M6P bind to the IGF-II/M6P receptor to different binding sites (6). By binding of M6P-containing lysosomal enzymes to the IGF-II/M6P receptor, the enzymes can be transported from the site of synthesis or from the extracellular space to the lysosomes (2). The functions of the IGF-II/M6P receptors in transportation of the enzymes and degradation of IGF-II are accomplished by trafficking of the receptors between the plasma membrane and intracellular membranes (2). Cellular events interfering with the receptor trafficking interfere with disposal of the lysosomal enzymes. It has been estimated that the majority of the IGF-II/M6P receptors are localized in the intracellular space, with about 10% of total receptors at the cell surface, in equilibrium with intracellular pools (7, 8). Although little is known about the mechanisms of regulation of receptor trafficking, IGF-I, IGF-II, epidermal growth factor, and phosphor 12-myristate 13-acetate (PMA) were shown, in certain cell types, to stimulate binding and endocytosis of the lysosomal enzymes (8–10). Moreover, insulin has been shown to induce redistribution of the IGF-II/M6P receptor to the cell surface (11, 12).

In the present study, we have investigated the effect of glucose on IGF-II binding to, and phosphorylation of, the IGF-II/M6P receptor in the rat insulinoma cell line RINm5F.

**Materials and Methods**

Recombinant IGF-II and IGF-I were generous gifts from Pharmacia, Uppsala, Sweden. Rat insulin was from Novo Nordisk A/S, Denmark. D(-)-Glucose, d-mannose 6-phosphate, 3-O-methylglucose, d-fructose, okadaic acid (OA), 12-O-tetradecanoylphorbol-13-acetate (TPA), and calphostin C were from Sigma. [125I]Orthophosphate was from DuPont NEN. Protein A-Sepharose CL-4B was from Pharmacia. Antiserum against rat IGF-II/M6P receptor was a generous gift from Dr. Peter Nissley, Metabolism Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD.

**Cell Culture**—The rat insulinoma cell line RINm5F, the insulin-secreting cell line HIT, and the human erythroleukemia cell line K562 were maintained in RPMI 1640 tissue culture medium supplemented with 5% (K562) or 10% fetal calf serum (RINm5F and HIT), 100 IU/ml penicillin, and 100 µg/ml streptomycin.

**Binding Assays**—Binding assays were performed as described previously (13). Cells were plated in 24-well culture dishes and grown to 80% confluence. Cells were washed four times with HEPES buffer containing 10 mM HEPES, 155 mM NaCl, 4.8 mM KCl, 1.7 mM MgSO4, 2.5 mM CaCl2, and 1.0 mM NaH2PO4, pH 7.4, followed by incubation of the cells for 30 min at 37 °C with [125I]IGF-II or -I, in the presence or absence of 100 nM IGF-II, 1 µM calphostin C, or 1 µM OA in HEPES buffer, 1% bovine serum albumin, pH 7.4, in the presence of various concentrations of glucose (0, 3, 10, or 20 mM), M6P (10 mM), d-fructose (10 mM), or 3-O-methylglucose (10 mM). After incubation, the cells were washed with ice-cold HEPES buffer and lysed in 0.1 M NaOH. To down-regulate PKC activity, cells were incubated in culture medium with 1 µM TPA (14) for 3 h before the binding assay. IGF-II binding to K562 cells was performed in cell suspension as described elsewhere (1).
Glucose-induced Increase in IGF-II Binding

**RESULTS**

Specific binding of 125I-IGF-II to RINm5F cells in the absence of glucose at 37 °C was 18.6%, which significantly increased to 21.7 and 25.7% at glucose concentrations of 3 and 10 mM, respectively (Fig. 1A). Further increase of the glucose concentration from 10 to 20 mM caused no change in the IGF-II binding. The specificity of the IGF-II/M6P receptor in the binding assays was evaluated by competitive inhibition studies, where the radiolabeled IGF-II bound to the cells was displaced by unlabeled IGF-II, but not by IGF-I and insulin (1) (data not shown). Scatchard analysis of the binding data (inset in A) revealed a parallel right shift induced by glucose and showed that glucose (10 mM) increased the number of IGF-II binding sites per cell from $4.3 \times 10^4$ to $6.1 \times 10^4$ without changing the affinity for IGF-II. Insulin did not significantly increase IGF-II binding at a concentration of 2 nM, which is about 50-fold higher than what is secreted from a similar number of RINm5F cells for 30 min at 37 °C in the presence of 20 mM glucose (data not shown). The stimulatory effect of glucose on IGF-II binding was also investigated in another insulin-secreting cell line HIT (Fig. 1B). Glucose increased IGF-II binding to HIT cells, and Scatchard analysis of the binding data revealed a significant and parallel right shift induced by glucose (inset in B). In addition, in the human erythroleukemia cell line K562, where receptors for IGF-II, but not for IGF-I, are present, glucose increased the 125I-IGF-II binding similar to that of RINm5F cells and HIT cells (Fig. 1C). Scatchard analysis of the binding data (inset in C) again showed a significant and parallel right shift induced by glucose. In contrast, no significant changes were found in 125I-IGF-I binding to the RINm5F cells in the presence or absence of 10 mM glucose (Fig. 1D).

Affinity cross-linking of RINm5F cells with 125I-IGF-II revealed a 250-kDa protein band, enhanced by 10 or 20 mM glucose, and displaced by 100 nM unlabeled IGF-II (Fig. 2). An additional protein band of about 80 kDa was also stimulated by glucose. However, unlabeled IGF-II failed to compete with labeled IGF-II for binding to the latter protein. Density analysis of the 250-kDa protein bands with a scanner connected to a computer revealed a 41% increase in the presence of 10 mM glucose compared with that with 0 mM glucose.

The glucose-induced increase in IGF-II binding was also obtained by fructose, but not by the unmetabolizable sugar 3-O-methylglucose (Fig. 3). In the absence of glucose, neither the protein phosphatase inhibitor, OA (1 μM) nor the protein kinase C inhibitor, calphostin C (1 μM), had a significant effect on IGF-II binding to the cells. However, calphostin C, but not OA, completely abolished the IGF-II binding stimulated by 10 mM glucose. A combination of OA with calphostin C did not show an additional effect (Fig. 4A). Down-regulation of PKC activity by TPA (1 μM, 3 h) prevented the stimulatory effect of glucose on IGF-II binding to the cells (Fig. 4B).

The phosphorylation state of the IGF-II/M6P receptor was evaluated after incubation of the cells for 5 min in the presence of different concentrations of glucose (Fig. 5). A major protein band of 250 kDa, precipitated by anti-rat IGF-II/M6P receptor...
antibodies, was phospho-labeled and was enhanced in density by glucose in a dose-dependent manner. Down-regulation of PKC activity with TPA inhibited the stimulatory effect of glucose. Density analysis of the IGF-II/M6P receptor protein bands revealed that the density, compared with that at 0 mM glucose, increased 124 and 283% at glucose concentrations of 3 and 10 mM, respectively.

**DISCUSSION**

The present study shows that glucose increases IGF-II binding to the IGF-II/M6P receptor. A PKC inhibitor, calphostin C, but not the protein phosphatase inhibitor OA, was able to prevent the glucose-induced increase in IGF-II binding. The increased IGF-II binding to the cells induced by glucose was due to an increased number of the IGF-II/M6P receptor at the cell surface, which was associated with an increased phosphorylation of the receptors.

The stimulatory effect of glucose on IGF-II binding to the cells can also be achieved by fructose, which is phosphorylated to fructose 6-phosphate in the cells, but not by the unmetabolizable sugar 3-O-methylglucose, suggesting a requirement of sugar metabolism in the glucose-stimulated binding. The fact that both fructose and glucose have stimulatory effects indicates that the early step of glucose metabolism, phosphorylation of glucose to glucose 6-phosphate catalyzed by glucokinase, is not required in this event.

The effect of glucose on IGF-II binding cannot be attributed to insulin secretion from the cells, since RINm5F cells have limited insulin content and do not respond to glucose with insulin release (16). In our hands, insulin release from the cells slightly increased when the glucose concentration was increased from 0 to 3 mM. No further increase was observed at concentrations above 3 mM. In addition, insulin added to the incubation medium had no effect on the IGF-II binding. Furthermore, when insulin was completely absent during the experiments, such as in the case of K562 cells, glucose stimulated IGF-II binding to the cells in the same manner as that in RINm5F cells.

Glucose-induced activation of PKC has been observed in pancreatic islets and β cells through generation of diacylglycerol (17, 18). A PKC-activating phorbol ester, phorbol 12,13-dibutyrate, enhanced IGF-II/M6P receptor distribution to the cell surface, an effect which was not altered by OA (5, 9). However, IGF-II-stimulated redistribution of the receptor was prevented by OA treatment (5), suggesting the involvement of two different regulatory pathways, namely, the phorbol ester-sensitive PKC pathway and the OA-sensitive protein phosphatase pathway, in the regulation of the IGF-II/M6P receptor distribution. The glucose-induced IGF-II binding to RINm5F cells was abolished by calphostin C, but not by OA, indicating that activation of PKC rather than inhibition of protein phosphatases was essential for the glucose-induced receptor distribution.

Insulin-stimulated redistribution of the IGF-II/M6P receptor to the plasma membrane is associated with a decreased internalization and phosphorylation state of the receptor (12, 19, 20), while the PKC-induced increase of receptors at the cell surface is associated with an increased receptor phosphorylation (9). In the present study, glucose enhanced the number of IGF-II/M6P receptors at the cell surface, an effect that was accompanied by an increased phosphorylation state of the receptors. The increased phosphorylation of the receptor cannot be attributed only to an increase in number of the receptors, since the receptor binding and the number of the IGF-II binding sites increased 30–40% at 10 mM glucose, compared with 0 mM glucose, as demonstrated in both receptor binding assays and affinity cross-linking studies, while 10 mM glucose caused a 3-fold increase in receptor phosphorylation. The inhibitory effect of down-regulation of PKC activity in the glucose-induced receptor phosphorylation suggests the involvement of PKC. The PKC-dependent manner indicates that phosphorylation of the IGF-II/M6P receptor induced by glucose is distinct from that by insulin (12, 20), but shares a similarity with phorbol

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ester (9). It is likely that generation of diacylglycerol by glucose metabolism leads to activation of PKC, resulting in an increased phosphorylation of the IGF-II/M6P receptor, and thereby increasing the concentration of receptors in the plasma membrane. This is especially interesting, given the fact that the IGF-II/M6P receptor may be involved in the regulation of insulin exocytosis in the pancreatic β cells (21).

REFERENCES
1. Zhang, Q., Hall, K., and Tally, M. (1996) Mol. Cell. Biochem. 154, 47–54
2. Roth, R. A., and Kiess, W. (1994) Growth Regul. 4, Suppl. 1, 31–38
3. Oh, Y., Muller, H. L., Neely, E. K., Lamson, G., and Rosenfeld, R. G. (1995) Growth Regul. 3, 113–123
4. Schmidt B., Kiecke-Siensen C., Waheed A., Braulke T., and von Figura K. (1995) J. Biol. Chem. 270, 14975–14982
5. Braulke, T., and Mieskes, G. (1992) J. Biol. Chem. 267, 17347–17353
6. Waheed, A., Braulke, T., Junghans, U., and von Figura, K. (1988) Biochem. Biophys. Res. Commun. 152, 1248–1254
7. Braulke, T., Gartung, C., Hasilik, A., and von Figura, K. (1987) J. Cell Biol. 104, 1735–1742
8. Braulke, T., Tippner, S., Neher, E., and von Figura, K. (1989) EMBO J. 8, 681–686
9. Hu, K.-Q., Backer, J. M., Sahagian, G., Feener, R. P., and King, G. L. (1990) J. Biol. Chem. 265, 13864–13870
10. Kornfeld, S. (1992) Annu. Rev. Biochem. 61, 307–330
11. Oka, Y., Rozeck, L. M., and Czech, M. P. (1985) J. Biol. Chem. 260, 9435–9442
12. Corvera, S., Roach, P. J., DePaoli-Roach, A. A., and Czech, M. P. (1988) J. Biol. Chem. 263, 3116–3122
13. Laurenzi, M. A., Sandberg Nordqvist, A.-C., Carlsson-Skwirut, C., Zhang, Q., and Sara, V. R. (1995) Neurosci. Res. Commun. 16, 37–43
14. Arkhammar, P., Nilsson, T., Welsh, M., Welsh, N., and Berggren, P.-O. (1989) Biochem. J. 264, 207–215
15. Pilch, P. F., and Czech, M. P. (1979) J. Biol. Chem. 254, 3375–3381
16. Praz, G. A., Halban, P. A., Wellheim, C. B., Blundell, B., Strauss, A. J., and Renold, A. E. (1983) Biochem. J. 210, 345–352
17. Wollheim, C. B., Dunne, M. J., Peter-Riesch, B., Bruzziote, R., Pozzan, T., and Petersen, O. H. (1988) EMBO J. 7, 2443–2449
18. Peter-Riesch, B., Patbi, M., Schlegel, W., and Wollheim, C. B. (1988) J. Clin. Invest. 81, 1154–1161
19. Corvera, S., Folannder, K., Clairmont, K. B., and Czech, M. P. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7567–7571
20. Corvera, S., Yagaloff, K. A., Whitehead, R. E., and Czech, M. P. (1988) Biochem. J. 250, 47–52
21. Zhang, Q., Tally, M., Larsson, O., Kennedy, R. T., Huang, L., Hall, K. and Berggren, P.-O. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6232–6237
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