Insulin Activation of Protein Kinase C: A Reassessment*

(Received for publication, February 4, 1991)

Perry J. Blackshear†, D. McNeill Haupt, and Deborah J. Stumpo‡

From the Howard Hughes Medical Institute Laboratories and the Section of Diabetes and Metabolism, Division of Endocrinology, Metabolism and Genetics, Department of Medicine and Biochemistry, Duke University Medical Center, Durham, North Carolina 27710

Although insulin is known to activate several protein serine/threonine protein kinases, its ability to activate protein kinase C remains controversial. We reinvestigated this question, taking advantage of several technical advances such as the development of fibroblast cell lines that overexpress normal human insulin receptors, and the development of antibodies to and expression vectors for the myristoylated, alanine-rich C kinase substrate (MARCKS) protein, a major cellular substrate for protein kinase C. In HIR 3.5 cells, a mouse 3T3 cell derivative that expresses about 6 × 10⁶ human insulin receptors/cell, insulin (70 nM for 10 min) stimulated phosphorylation of the MARCKS protein by approximately 2-fold (p < 0.005). This phosphorylation was not further increased by different times of insulin exposure, different insulin concentrations, or longer periods of serum deprivation. The insulin stimulation represented about 14% of the response to phorbol 12-myristate 13-acetate and about 17% of the response to 10% fetal calf serum. No significant stimulation of MARCKS protein phosphorylation was seen in four other insulin-sensitive cell lines, in which insulin is known to activate other protein serine/threonine kinases: HIRC-B, BC3H-1, 3T3-L1 adipocytes, and H35 rat hepatoma cells made to stably express the MARCKS protein. In these four cell lines, serum and/or phorbol 12-myristate 13-acetate exerted a large stimulatory effect on MARCKS protein phosphorylation. We conclude that insulin may activate protein kinase C to a minor extent in certain cell types that vastly overexpress insulin receptors; however, we believe that this effect of insulin is unlikely to be of physiological importance.

Most if not all of the effects of insulin and several other polypeptide growth factors are mediated by the initial activation of the protein tyrosine kinase activities of the respective cell surface receptors. Through the mediation of largely unknown intermediate reactions, the activated insulin receptor kinase leads to the activation of a number of protein serine/threonine kinases; these activated enzymes are presumably early steps in the activation of many cellular processes that mediate the myriad effects of insulin on cells. Among the serine/threonine kinases known to be activated by insulin are the ribosomal protein S6 kinases (1–3), the microtubule-associated protein 2 kinases (4–6), casein kinase 2 (7), the proto-oncogene Raf-1 kinase (8, 9), and several others.

One prominent growth factor-activated kinase is protein kinase C (PKC). However, the question of whether this kinase is activated by insulin remains unresolved. On one hand are studies from our group and other groups in which protein kinase C activation was assessed by investigating the phosphorylation of the myristoylated, alanine-rich C kinase substrate, or MARCKS protein, a prominent and apparently specific substrate for protein kinase C in intact cells. In studies in several cell types, insulin failed to stimulate the phosphorylation of the MARCKS protein in cells in which potent tumor-promoting phorbol esters such as phorbol 12-myristate 13-acetate (PMA), synthetic cell-permeable diacylglycerols, serum or other growth factors such as platelet-derived growth factor all stimulated a large increase in MARCKS protein phosphorylation (10–12). In addition, most effects of insulin were normal in several cell types from which PKC had been almost completely removed by prolonged incubation with high concentrations of PMA, a process known as “down-regulation” that appears to be due to accelerated proteolysis of the kinase (8, 10, 12–19).

On the other hand are studies from several groups in which insulin activation of PKC was inferred from experiments in which insulin appeared to cause “translocation” of PKC from one cellular compartment to another; in most cases this was from a cytosolic to a particulate fraction, whereas in others there appeared to be a net increase in PKC activity in both cytosolic and particulate fractions (reviewed in Refs. 20 and 21). These and other groups have also called into question the validity of the down-regulation studies, given the recent discovery of PKC subtypes that appear to be more resistant to down-regulation (such as PKC-ζ), and which might be preferentially activated by insulin in some cell types (22).

We decided to reassess the possibility that insulin could activate PKC because of some recent technical advances that might allow us to overcome certain problems with our earlier studies. Examples of these problems include the relatively low number of insulin receptors present in 3T3-L1 fibroblasts used in one study (10), and similar low numbers in the Swiss 3T3 cells used by Rozengurt and colleagues (11) in a study that reached similar conclusions; the fact that the 3T3-L1 adipocytes used in one study expressed relatively little MARCKS protein, despite expressing many more insulin receptors than their fibroblast precursors (10); the fact that H35 or H4IIE rat hepatoma cells, which express large numbers of insulin receptors, express no MARCKS protein (14); and the necessity to use two-dimensional electrophoresis to

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† Investigator of the Howard Hughes Medical Institute. To whom all correspondence should be addressed: Box 3897, Duke University Medical Center, Durham, N.C. 27710.

‡ Associate of the Howard Hughes Medical Institute.

1 The abbreviations used are: PKC, protein kinase C; MARCKS, myristoylated alanine-rich C kinase substrate protein; PMA, phorbol 12-myristate 13-acetate; TTP, Tris-tetraprolin.
evaluate MARCKS protein phosphorylation, a technique that does not lend itself to the statistical comparison of large numbers of samples. The technical advances that we hoped to make in this re-evaluation productive include the development of rodent fibroblast lines that express large numbers of human insulin receptors and continue to express large quantities of the MARCKS protein (23–25), the development of antibodies to this protein, which permits the convenient comparison of larger numbers of samples (26), and the development of eucaryotic expression vectors for the bovine MARCKS protein (27), permitting the development of H35 rat hepatoma cell lines that express the protein. We used these technical advances to re-evaluate the question of whether insulin activates PKC in five cell lines known to be very sensitive to insulin. We evaluated PKC activation by studying the sine qua non of protein kinases, the phosphorylation of specific substrates; this was used to overcome the problems associated with assessment of changes in PKC subcellular localization, which are not necessarily reflective of activation (28–30).

**EXPERIMENTAL PROCEDURES**

The cell lines used in this study are described in the following publications: HIR 3.5 (24); HIRC-B (25); BC3H-1 cells (31), differentiated as described (19); 3T3-L1 adipocytes (32), differentiated as described (10); and H35 (33). The H35 cells used in this study expressed the bovine MARCKS protein; this cell line was created by transfecting the parental cells with an expression vector based on the mouse β-actin promoter (34) and the bovine MARCKS cDNA (27). The expression vector also contains the gene for neomycin resistance (34). Following transfection using Lipofectin (Life Technologies, Inc., Gaithersburg, MD), the cells were treated for 2–3 weeks with G418, resistant colonies were evaluated for expression of the MARCKS protein and mRNA, and the two clones expressing the greatest amount of mRNA and protein were expanded for further use. The clones were evaluated for MARCKS protein expression by Western blotting with an antibody directed at the bovine MARCKS protein (55; a generous gift from Dr. Angus C. Nairn, the Rockefeller University, New York); clone H15 expressed the greatest amounts of bovine MARCKS protein. This protein, when expressed in these cells, could be phosphorylated in vitro by protein kinase C and in intact cells in response to phorbol esters (data not shown). The clone used in this study was H15.

Except where otherwise specified, the culture conditions and method for serum deprivation were as described for the individual cell lines: HIR 3.5 (17); HIRC-B (18); BC3H-1 (12); 3T3-L1 adipocytes (14). Cell labeling with 32P and immunoprecipitation were carried out as described (26), using an antisera raised against a synthetic amino-terminal MARCKS peptide that is identical in the MARCKS proteins from cow, chicken, man, and rat hepatoma cell lines that express the protein. When the data were analyzed densitometrically (Fig. 1B), the stimulation by insulin was approximately 2-fold compared to control; the difference was statistically significant (p < 0.005). In the same experiment, the PMA stimulation of MARCKS protein phosphorylation was about 10-fold, and the stimulation by serum was about 8-fold. Thus, the stimulation by insulin was about 14% of the PMA and 17% of the serum stimulation. We should note that the insulin-stimulated MARCKS protein phosphorylation shown in Fig. 1 represents the greatest degree of stimulation seen in at least five separate experiments, although the difference between control and insulin was statistically significant on other occasions (see below). When the results of these five experiments were averaged, the insulin stimulation was 1.6 ± 0.2-fold (mean ± S.E.), whereas the PMA stimulation was 9.0 ± 2.6-fold (data not shown).

We modified this experiment in several ways in an attempt to maximize the insulin stimulation of MARCKS protein phosphorylation. First, the time of insulin exposure was varied between 30 s and 20 min. At no time was the stimulation greater than that achieved at 10 min (data not shown). Second, the insulin concentration at 10 min was varied between 0.007 and 70 nM; again, 70 nM achieved maximum stimulation (not shown). We tried to mimic the insulin effect with insulin-like growth factor I; however, exposure of the cells to 7 and 70 nM insulin-like growth factor I for 10 min was, if anything, less effective than insulin at the same concentration at promoting MARCKS protein phosphorylation (not shown). Finally, we tried 40 h of serum deprivation instead of the 18 h routinely used in our experiments; insulin (70 nM for 10 min) was also able to stimulate MARCKS protein phosphorylation to a significant degree (22% stimulation; p < 0.05; see Fig. 3A below). No other maneuvers resulted in a greater degree of insulin-stimulated MARCKS protein phosphorylation than that achieved at 10 min with 70 nM insulin.

We next performed identical experiments in four additional insulin-sensitive cell types. HIRC-B cells are rat-1 fibroblast derivatives that express about 1.25 × 10^6 normal human insulin receptors/cell (25). Previously noted insulin responses in these cells have included stimulation of glucagon transport and glycogen synthesis (25), tyrosine phosphorylation of cellular proteins (36), activation of Raf-1 kinase (8), induction of the c-fos, EGR-1 (18), and TTP (17) genes, and others. In these cells, insulin (70 nM for 10 min) had no significant effect on MARCKS protein phosphorylation, even though both PMA and serum stimulated massive increases in the phosphorylation of this protein in the same cells (increases of 24- and 19-fold, respectively; Fig. 2, A and B). Results in three other cell lines are summarized in Fig. 3. Fig. 3A shows the results in HIR 3.5 cells alluded to above in which the cells were serum-deprived for 40 h before 32P-labeling and exposure to insulin or other agonists. In Fig. 3A and B, the results of an experiment in 3T3-L1 adipocytes that was performed under identical conditions to those shown in Figs. 2 and 3. These cells have been shown to respond to insulin in a number

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**RESULTS AND DISCUSSION**

Initial studies were performed in HIR-3.5 cells, a mouse fibroblast cell line that expresses approximately 6 × 10^6 normal human insulin receptors/cell (24). These cells are very sensitive to insulin; responses noted include stimulation of glycogen synthesis, (24), activation of the Raf-1 protein kinase (8), induction of ornithine decarboxylase (18), stimulation of transcription of the Tris-tetraprolin (TTP) gene (17) and c-fos, and others. When these cells were stimulated for 10 min with either buffer control or insulin (70 nM), there appeared to be a modest increase in 32P-labeling of the MARCKS protein (Fig. 1A). This was considerably less than that achieved with 1.6 μM PMA or 10% (v/v) fetal calf serum, both stimuli known to activate PKC near maximally in 3T3 cells and their derivatives (10, 11). When the data were analyzed densitometrically (Fig. 1B), the stimulation by insulin was approximately 2-fold compared to control; the difference was statistically significant (p < 0.005). In the same experiment, the PMA stimulation of MARCKS protein phosphorylation was about 10-fold, and the stimulation by serum was about 8-fold. Thus, the stimulation by insulin was about 14% of the PMA and 17% of the serum stimulation. We should note that the insulin-stimulated MARCKS protein phosphorylation shown in Fig. 1 represents the greatest degree of stimulation seen in at least five separate experiments, although the difference between control and insulin was statistically significant on other occasions (see below). When the results of these five experiments were averaged, the insulin stimulation was 1.6 ± 0.2-fold (mean ± S.E.), whereas the PMA stimulation was 9.0 ± 2.6-fold (data not shown).

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**Footnote:** M. W. Roe, R. K. Malik, and P. J. Blackshear, submitted for publication.
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A Control  Insulin

B

FIG. 1. Effect of insulin, PMA, and serum on MARCKS protein phosphorylation in HIR 3.5 cells. Serum-deprived, confluent HIR 3.5 cells were labeled with $^{32}$P and then exposed for 10 min to buffer alone (C), insulin (70 nM; I), PMA (1.6 μM), or fetal calf serum (FCS; 10% (v/v)). The cells were then homogenized, centrifuged, and equal amounts of trichloroacetic acid-precipitable radioactivity were used for immunoprecipitation with an anti-MARCKS amino-terminal peptide antiserum, at a dilution of 1:200. Equal volumes of sample from the immunoprecipitates were then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. In A is shown the original autoradiograph; each lane contains sample from a single plate of cells. The position of the $M_r$, 80,000 MARCKS protein is indicated by the arrow; its migration position in the same gel type (7.2% acrylamide Laemmli gel) relative to proteins of known molecular weight can be found in Ref. 26. In B are shown the results of densitometric evaluation of the autoradiograph, as well as the results of a statistical comparison between the mean control and insulin-stimulated samples. The bars indicate ± standard deviation. Note that although the four control and four insulin samples were subjected to electrophoresis together, each lane is derived from a separate plate of cells.

of ways, including stimulation of glucose transport and oxidation (37), ribosomal protein S6 phosphorylation and activation of S6 kinase (10), induction of the genes for c-fos (13) and TTP (17), and others. Insulin had no effect on MARCKS protein phosphorylation, even though PMA and serum stimulated its phosphorylation by 9- and 6-fold, respectively. These results agree with those of our previous study, in which MARCKS protein phosphorylation in these cells was evaluated by two-dimensional gel electrophoresis (10).

In Fig. 3C are shown the results of an identical experiment in H35 cells (clone H15), an insulin-sensitive rat hepatoma cell line (33) that in this case expressed the bovine MARCKS protein. The parental H35 cells have been shown to respond to insulin by increases in DNA synthesis and mitogenesis (33), activation of S6 kinase (14), induction of ornithine decarboxylase (14), stimulated transcription of the c-fos gene (38, 39), inhibited transcription of the gene encoding phosphoenolpyruvate carboxykinase (15), activation of the Raf-1 (8) and S6 kinases (14), and others. Once again, insulin had no effect on MARCKS protein phosphorylation, in the same experiment in which PMA stimulated phosphorylation of the protein by 27-fold, and serum stimulated its phosphorylation 8-fold. These results agree with our previous study in these cells, in which MARCKS protein phosphorylation was evaluated by two-dimensional gel electrophoresis (12). They do not agree with the results of a study by Vila et al. (44) in the same cells; however, examination of their published figures indicates that the protein described as the “80-kDa” protein in their study almost certainly does not correspond to the MARCKS protein.

To summarize these results, insulin failed to stimulate MARCKS protein phosphorylation to a detectable degree in four of the five cell lines tested, despite large increases in its phosphorylation caused by exposure to PMA and serum in most cases. In one cell line, HIR 3.5 cells, insulin stimulated a modest increase in the phosphorylation of the protein that was statistically significant in two independent experiments; however, insulin was still only about 14% as effective as PMA and 17% as effective as serum in stimulating MARCKS
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FIG. 2. Effect of insulin, PMA, and serum on MARCKS protein phosphorylation in HIRC-B cells. All aspects of this figure are the same as described in the legend to Fig. 1, except that the cells used were HIRC-B cells. NS, not statistically significant (p > 0.05); FCS, fetal calf serum.

FIG. 3. Effect of insulin, PMA, and serum on MARCKS protein phosphorylation in HIR 3.5 cells, 3T3-L1 adipocytes, H35 rat hepatoma cells (clone H15), and BC3H-1 cells. All aspects of this figure are the same as described in the legends to Figs. 1 and 2, except that the HIR 3.5 cells were deprived of serum for 40 h in A, and in D, the control and fetal calf serum (FCS) samples were from a different gel than the control, insulin, and PMA samples. In A-D, the bars for the control and insulin samples represent the means ± S.D. of values from five plates of cells for each treatment; the bars for the PMA and fetal calf serum samples represent results from a single plate of cells.

protein phosphorylation in the experiment in which its greatest effect was seen (Fig. 1).

These cell lines have also been used in experiments in which the effect of insulin was assessed in cells exposed overnight to high concentrations of PMA in order to down-regulate protein kinase C. Table I summarizes data from our laboratory, using a constant down-regulating procedure in which the cells are exposed to 16 μM PMA in serum-free medium for 16 h. In all cases, the extent of down-regulation was monitored by measurement of protein kinase C activity; in some cases, this was supplemented by measurement of PKC immunoreactivity or specific binding of phorbol esters. The extent of down-regulation varied from cell type to cell type; most noteworthy is the relative lack of effectiveness of this technique in the 3T3-L1 fat cells, in which we postulated that the high content of neutral lipid in the cells might bind the PMA avidly, preventing adequate proteolysis of PKC (10). Despite this variability in the extent of down-regulation, all of the insulin responses measured in all of the cells tested were essentially normal in the down-regulated cells, whereas the responses to PMA were, in all cases, completely abrogated.

Taken together, the studies described here concerning the insulin-stimulated phosphorylation of the MARCKS protein, and the previous studies of insulin action in PKC down-regulated cells, suggest the following conclusions. Under certain circumstances, as in the case of the HIR 3.5 cells expressing 6 × 10⁶ insulin receptors/cell, insulin can activate PKC to a modest degree; however, in our view, insulin activation of PKC is unlikely to be of physiological significance.

Several types of criticism can be applied to both the present studies and previous studies involving down-regulation of PKC. One is that the MARCKS protein may not be a sub-
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Effect of PKC depletion on various responses to insulin

In all cases, the extent of PKC down-regulation was assessed as described in Ref. 60. The abbreviations used and descriptions of the cell types are described in the text and in the appropriate references. Other abbreviations used are: 22K, an M, 22,000 protein whose phosphorylation is stimulated by insulin; ODC, ornithine decarboxylase; PEPCK, phosphoenol pyruvate carboxykinase.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Extent of PKC down-regulation</th>
<th>Insulin or PMA response</th>
<th>Effect of down-regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3T3-L1 fibroblast</td>
<td>~100%</td>
<td>Phosphorylation of S6</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activation of S6 kinase</td>
<td>Normal</td>
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<tr>
<td></td>
<td></td>
<td>Phosphorylation of 22K</td>
<td>Normal</td>
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<tr>
<td></td>
<td></td>
<td>Induction of TTP</td>
<td>Normal</td>
</tr>
<tr>
<td>3T3-L1 adipocyte</td>
<td>~70%</td>
<td>Phosphorylation of S6</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activation of S6 kinase</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phosphorylation of 22K</td>
<td>Normal</td>
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<td></td>
<td></td>
<td>Induction of c-fos</td>
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<td></td>
<td></td>
<td>Activation of c-fos</td>
<td>Normal</td>
</tr>
<tr>
<td>HIRC-B cells</td>
<td>~100%</td>
<td>Phosphorylation of S6</td>
<td>Normal</td>
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<tr>
<td></td>
<td></td>
<td>Induction of c-fos</td>
<td>Normal</td>
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<tr>
<td>HIR 3.5 cells</td>
<td>~95%</td>
<td>Phosphorylation of Raf-1 kinase</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Activation of Raf-1 kinase</td>
<td>Normal</td>
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<td></td>
<td></td>
<td>Phosphorylation of ODC</td>
<td>Normal</td>
</tr>
<tr>
<td>CHO-T cells</td>
<td>~94%</td>
<td>Phosphorylation of S6</td>
<td>Normal</td>
</tr>
<tr>
<td>BC3H-1 cells</td>
<td>~100%</td>
<td>Phosphorylation of S6</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Which decreases the cellular amounts of the other isozymes due to an increased rate in their proteolysis (52, 28); however, this does not seem to be the case in COS cells expressing PKC-ε, in which this isozyme is down-regulated normally or even more rapidly than the endogenous PKC species. The failure to detect residual PKC enzyme activity after down-regulation has been attributed to differences in the substrate specificity of the calcium-independent PKC subspecies, which appear to prefer substrates other than the more commonly used PKC substrate, histone (46, 47). However, in many of the down-regulation experiments performed by our group and others, the extent of the down-regulation has been monitored not only by enzyme activity measurements but also by assay of immunoreactive PKC (with a polyclonal antiserum directed at all brain PKC activities) and specific phorbol-ester binding (10). Even if an isozyme of PKC were to escape detection by the Western blots performed with antibody raised against a pool of PKC that would not have access to the substrate.

Similar criticisms can and have been directed at the down-regulation experiments. For example, it has been suggested that the calcium-independent isozymes of PKC, exemplified by PKC-ε, may be relatively unresponsive to down-regulation by prolonged treatment with phorbol esters (51), a maneuver which decreases the cellular amounts of the other isozymes due to an increased rate in their proteolysis (52, 28); however, this does not seem to be the case in COS cells expressing PKC-ε, in which this isozyme is down-regulated normally or even more rapidly than the endogenous PKC species. The failure to detect residual PKC enzyme activity after down-regulation has been attributed to differences in the substrate specificity of the calcium-independent PKC subspecies, which appear to prefer substrates other than the more commonly used PKC substrate, histone (46, 47). However, in many of the down-regulation experiments performed by our group and others, the extent of the down-regulation has been monitored not only by enzyme activity measurements but also by assay of immunoreactive PKC (with a polyclonal antiserum directed at all brain PKC activities) and specific phorbol-ester binding (10). Even if an isozyme of PKC were to escape detection by the Western blots performed with antibody raised against a mixture of rat brain PKC subtypes, all subtypes, essentially by definition, bind phorbol esters; disappearance of specific, high affinity phorbol ester binding represents confirmation that all forms of PKC have decreased under the conditions employed.

What is one to make, then, of the numerous studies in which insulin activation of PKC has been inferred from changes in subcellular compartmentation of the kinase (reviewed in Refs. 20, 21; see also Refs. 53, 54)? We believe that studies in which activation of PKC is inferred from changes in its subcellular distribution are liable to be misinterpreted for a number of reasons. The first is the theoretical point that
PKC, so far as is known, is activated allosterically rather than by autophosphorylation, covalent modification or subunit dissociation, all modes by which other protein kinases are rendered stably active. This means that, once cells are broken open for assay of PKC activity, the allosteric activators would be expected to dissociate from the kinase, and thus the kinase activity in a given cellular fraction would represent the amount of enzyme protein present rather than the activity state of the protein before cell disruption. A second general point is that studies of PKC translocation have been difficult to perform and reproduce (see Ref. 30, for review). Third, several studies have found changes in PKC subcellular localization in response to stimuli that do not seem to activate PKC; for example, Cambier et al. (55) found that dibutyryl cAMP caused redistribution of PKC to the nucleus of B lymphocytes. These studies suggest the possibility that insulin could change the subcellular localization or membrane association of PKC without affecting its activation state. In this regard, insulin is capable of "translocating" several proteins from intracellular locations to the plasma membrane, including the glucose transporter (56), the insulin-like growth factor 2 receptor (57), and the transferrin receptor (58). Fourth, certain isoenzymes of PKC apparently can be translocated to sites other than the plasma membrane upon activation. For example, Mochly-Rosen et al. (59) found that activation of PKC in cardiac myocytes and fibroblasts led to translocation of at least one PKC isoenzyme to myofilaments and microfilaments, respectively. Thus, the reported effects of insulin on translocation of PKC to cellular "particulate fractions" conceivably could be due to stimulated association of PKC with cellular components such as microfilaments. Finally, redistribution of PKC within the cell is sometimes impossible to demonstrate in response to stimuli known to activate the kinase (60). Because of these problems with studies of PKC translocation, we feel strongly that the best and most reliable means of demonstrating whether a given agonist activates PKC is to evaluate phosphorylation of its specific substrates in intact cells.

Such substrates are not, of course, limited to the MARCKS protein. For example, of about 1000 phosphoproteins identified in 3T3 cells by "giant" two-dimensional gel electrophoresis, PMA stimulated the phosphorylation of about 120. Although many of these are undoubtedly phosphorylated by secondary or even tertiary kinases, it seems likely that many if not most of these phosphoproteins are direct substrates for PKC. Any one of these proteins, or others in different cell types, could theoretically be used to assess PKC activation in intact cells, provided it meets the criteria of Krebs and Beavo (61) or the modification of these criteria suggested for PKC substrates (62). However, the MARCKS protein serves as a convenient marker of PKC activation in many cell types for a number of reasons: 1) it is phosphorylated within seconds of PKC activation; 2) the magnitude of the increase in phosphorylation is large, making it a sensitive index of PKC activation; 3) so far as is known, it is a specific substrate for PKC in intact cells; 4) the primary structures of the protein and its phosphorylation sites are known; and 5) it can be separated readily from other cellular phosphoproteins by two-dimensional electrophoresis and/or immunoprecipitation.

Based on the studies described here, we conclude that insulin can activate PKC to a modest extent in certain cells that express enormous numbers of insulin receptors. Whether the dicyglycerol that mediates this activation is derived from inositol phospholipids or is synthesized de novo is not known.

H. B. Sadowski, R. M. Levenson, and D. A. Young, unpublished data.

Acknowledgments—We are very grateful to the following colleagues for the cell lines used in this study: Drs. Jonathan Whittaker (HIR 3.5 cells), Drs. Jerold Olefsky, Donald McLain, and Axel Ullrich (HIRC-B cells). John Koontz (H35 cells), and Mary Standaert and Robert Pollet (BC3H-1 cells). We also thank Dr. Larry Kedes for the β-actin/neoeucaryotic expression vector, Dr. Angus C. Nairn for the bovine MARCKS antibody, and Jane S. Tuttle for technical assistance.

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