Irradiation of erythrocyte ghosts in the presence of [3H]forskolin resulted in a concentration-dependent, covalent incorporation of radiolabel into several of the major membrane protein bands. Most of the incorporation occurred in four regions of the gel. Peak 1 (216 kDa) was a sharp peak near the top of the gel in the region corresponding to spectrin. Peak 2 appeared to be associated with band 3 (89 kDa), while a third peak occurred around the position of band 4 (76 kDa). The fourth region of labeling was a broad area between 43–75 kDa which corresponds to the region of the glucose transporter. Forskolin labeling of this region was inhibited by cytochalasin B and D-glucose, but not l-glucose. Extraction of extrinsic membrane proteins resulted in a loss of radiolabeled protein from the 216- and 76-kDa regions. Treatment of membranes labeled with either cytochalasin B or forskolin with endo-β-galactosidase resulted in identical shifts of the 43 to 75-kDa peaks to 42 kDa. Similarly, trypsinization of membranes photolabeled with either cytochalasin B or forskolin resulted in the generation of a 17-kDa radiolabeled fragment in both cases. Photoincorporation of [3H]cytochalasin B into the glucose transporter was blocked in a concentration-dependent manner by unlabeled forskolin.

The glucose transport system of mammalian cells has been the subject of intensive investigation in the past few years (for reviews see Refs. 1–3). In spite of recent important advances in elucidating the structure of this protein (4), little is known concerning the mechanisms of regulation of this transport system either by hormone action or by metabolic feedback. Inhibitors of transport which interact directly with the transporter protein have proven to be valuable aids in providing important information about this system (5–21). In this regard, cytochalasin B has proven to be particularly suitable for this approach since under the proper conditions it can be used as a highly specific photoaffinity probe of the glucose transporter in a number of cell types (5–9, 11–18). In addition, other photoactive or chemical agents have been developed recently for use as probes of this transport protein (10, 19–21). The identification of new agents which specifically interact with the glucose transporter protein is therefore a useful approach which may lead to new tools for probing sites potentially involved in the regulation of transport activity.

* This work was supported by National Institutes of Health Grants AM 36855 and HL 36070. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom all correspondence should be addressed.

The diterpene forskolin is known to be a powerful activator of adenylyl cyclase in cells (22). It has also been reported to inhibit glucose transport in adipocytes (23), erythrocytes (24), and cardiomyocytes (25). While several reports have suggested indirectly that this inhibitory effect on transport was independent of changes in cell cAMP levels (23, 25, 26), the recent study on erythrocytes by Sergeant and Kim (24) has provided convincing evidence that forskolin indeed inhibits glucose transport, as well as glucose-sensitive cytochalasin B binding to the erythrocyte membrane, via a cAMP-independent mechanism.

In the present report we have investigated the interaction of [3H]forskolin with human erythrocyte membrane proteins in a more direct manner. By using methods similar to those for photoincorporation of [3H]cytochalasin B in these membranes (12–14), we have been able to demonstrate the photolabeling of at least four membrane polypeptides by [3H]forskolin, one of which appears to be the red cell glucose transporter.

EXPERIMENTAL PROCEDURES

Materials—[3H]Cytochalasin B (15 Ci/mmol) was obtained from Amerham Corp. while [3H]forskolin (30 Ci/mmol) was from Du Pont New England Nuclear. Molecular weight marker was purchased from Bio-Rad and included myosin (200 kDa), phosphorylase B (92.5 kDa), bovine serum albumin (68.3 kDa), ovalbumin (45 kDa), soybean trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa). Trypsin (Type III) was obtained from Sigma and endo-β-galactosidase from Miles Laboratories (Elkhart, IN). Forskolin was purchased from Behring Diagnostics. All other reagents were purchased from Sigma. Outdated blood was provided by the American Red Cross, Missouri-Illinois Regional Blood Services, St. Louis, MO.

Preparation of Plasma Membranes—Washed human erythrocytes and membrane ghosts were prepared by the method of Steck and Kant (27). Ghosts membranes in 5 mM sodium phosphate buffer, pH 8 (5P8 buffer) were isolated by centrifugation at 17,400 g for 15 min in a Sorvall SS-34 rotor and washed 3–4 times with 5P8 buffer. All procedures were carried out at 4 °C unless noted otherwise.

Protein-depleted Membranes—Plasma membranes were depleted of extrinsic proteins using the procedure reported by Sogin and Hinkle (28). Briefly, 1 volume of plasma membranes (3–4 mg/ml) was mixed with 3 volumes of 0.1 mM Na2EDTA, pH 11.2, solution on ice without stirring for 15 min. Following this incubation the pellet fraction was obtained by centrifugation at 27,100 × g for 15 min in a Sorvall SS-34 rotor and washed 3–4 times with 5P8 buffer. All procedures were carried out at 4 °C unless noted otherwise.

Protein-depleted Membranes—Plasma membranes were depleted of extrinsic proteins using the procedure reported by Sogin and Hinkle (28). Briefly, 1 volume of plasma membranes (3–4 mg/ml) was mixed with 3 volumes of 0.1 mM Na2EDTA, pH 11.2, solution on ice without stirring for 15 min. Following this incubation the pellet fraction was obtained by centrifugation at 27,100 × g for 15 min in a Sorvall SS-34 rotor and washed 3–4 times with 5P8 buffer. All procedures were carried out at 4 °C unless noted otherwise.

Photoaffinity Labeling—Forskolin photoincorporation into erythrocyte ghosts was performed essentially as cytochalasin B photolabeling done previously (8, 14). Tritiated forskolin in ethanol was added to 10 ml Pyrex tubes and dried down under nitrogen. It was resuspended in 106 µl of 5–10 mM phosphate buffer, pH 7.7–7.5, containing ghost membranes at a protein concentration of 2 mg/ml and incubated for 15 min on ice. The concentrations of [3H]forskolin during photolysis are stated in the legends and were determined in most cases by sampling 3–5 µl for scintillation counting immediately prior to photolysis. The membrane solutions in Pyrex tubes were
photolysed for 30 s in ice water at 10 cm from a 1-kilowatt high pressure mercury vapor lamp (AH-5, Advanced Radiation, Santa Clara, CA). Following photolysis samples were diluted with 10 ml of 10% TCE buffer, transferred to 15 ml Cori tubes, and centrifuged at 27,100 g for 15 min as described above. The resulting pellet was resuspended to a final concentration of about 1 mg/ml in electrophoresis sample buffer.

Conditions for photolabelling of membranes with [3H]cytochalasin B were the same as those described above with the addition of cytochalasin E to prevent nonspecific binding. Cytochalasin E and [3H]cytochalasin B were dried down in Pyrex tubes and resuspended as above. The [3H]cytochalasin E concentrations during photolysis were 180 μM while the [3H]cytochalasin B concentrations stated in the legends and were all determined by sampling as above.

Photolabeling studies in the presence of various inhibitors of photoincorporation were performed essentially as described above with the following modifications. With D- and L-glucose studies, the membranes were incubated for 15 min at 4°C in the presence of the radioligand and appropriate sugar (500 μM, added from a 1× stock in TCE buffer) followed by photolysis. For the studies involved in Fig. 8, either solid glucose or appropriate stock solutions of D-glucose were used in order to obtain the final concentrations depicted in the figure. The results in Fig. 8 are presented as percent inhibition of [3H]forskolin incorporation into ghost membranes based on control values in the presence of 0.56 M L-glucose (0% inhibition).

For cytochalasin inhibition of forskolin incorporation, [3H]forskolin was first added to the photolysis tube and dried under nitrogen. Membranes (100 μl) were then added to the tube and mixed by vortex. This was followed by the addition of 2 ml of either cytochalasin B or E (in ethanolic stock). The membrane solution was mixed and incubated for 15 min prior to photolysis.

Inhibition of [3H]cytochalasin B incorporation into ghosts by unlabeled forskolin (Fig. 7) were performed as follows. [3H]Cytochalasin B was first dried down in the photolysis tube followed by the addition of forskolin in MeSO2 at the appropriate concentration. The final concentration of MeSO2 was 1% in all cases following the addition of membranes (1 mg/ml). The mixture was incubated for 15 min on ice. The final volume for photolysis was 0.5 ml.

Enzymatic Digestions—Digestions were performed in postphotolysis solutions containing whole or protein-depleted ghosts. The approximate membrane protein concentrations were 2 mg/ml for trypsin and 4 mg/ml for glycosidase incubations. Trypsin digestion was performed by adding trypsin to a final concentration of 50 units/ml and incubation for 30 min at 37°C. Trypsin digestions were terminated by adding appropriate buffer containing soybean trypsin inhibitor to a final concentration of 125 units/ml.

Endo-β-galactosidase digestions were performed at a final enzyme concentration of 400 units/ml for 60 min at 37°C. The reaction was terminated by the addition of 2× concentrated electrophoresis sample buffer.

Electrophoresis—Electrophoresis was performed on 12% gels using the modified Laemmli buffer system of Gianlian et al. (29). The sample buffer consisted of 3% sodium dodecyl sulfate, 1 mM EDTA, 10% glycerol, 0.005% bromphenol blue, 2.5 mM dithiothreitol, 62.5 mM Tris-HCl, pH 6.8 (all at final concentrations). The amount of solubilized protein loaded in the gel lanes varied from 70–120 μg. Following electrophoresis, gels were fixed, stained, sliced, and assayed by scintillation counting as previously described (13). Gel slices were normalized to R values relative to the migration of the dye fronts to allow for comparison of gels of different lengths. In some cases the dpn or peak heights were normalized to spectrin labeling to account for differences in load or nonspecific labeling.

Other Procedures—Protein determinations were performed according to the modified Lowry procedure reported by Peterson (30).

RESULTS

Recent work by Sergeant and Kim (24) on forskolin inhibition of 3-O-methylglucose transport in erythrocytes suggested that forskolin was inhibiting transport by interacting directly with the glucose transporter rather than via a camp-dependent mechanism. Our own experiments in labeling this transport protein in red cells with cytochalasin B led us to suggest that cytochalasin B was incorporated into the transporter via direct photoactivation of residues on the protein itself (14). The studies by Deziel et al. (31) presented evidence which strongly suggested that photolabelling with cytochalasin B indeed proceeds via activation of an aromatic amino acid residue on the transporter protein. We, therefore, postulated that if forskolin competed with cytochalasin B binding, as suggested by others (24), that photolabelling of the transporter in the presence of [3H]forskolin might result in ligand incorporation via a mechanism similar to that of cytochalasin B. This was found to be the case.

Photoincorporation of [3H]Forskolin into Erythrocyte Membranes—Human erythrocyte ghosts were first incubated for 15 min with [3H]forskolin in the presence of either 500 mM D- or L-glucose. Following this initial incubation the membrane suspension was photolysed as described under "Experimental Procedures." Fig. 1 shows a typical electrophoretic profile of photoincorporation of radiolabel into the membrane polypeptides. In the presence of L-glucose, four polypeptide regions of the gel exhibited significant incorporation of [3H]forskolin, a high molecular mass peak of approximately 216 kDa, two labeled membrane components of apparent mass 89 kDa and 76 kDa, and a third broad region of labeling in the gel between 43 and 75 kDa (it should be noted that these molecular mass estimations are based on regression analysis of standard markers; however, the migration of the membrane proteins is dependent upon the total acrylamide concentration as well as the percent cross-linking of the gel system). Comparison of the radiolabeled regions with both molecular weight markers and Coomassie Blue-staining patterns obtained prior to slicing the gels indicated that the labeled protein in the high molecular mass region appeared to correspond to the migration of spectrin band 2 which is a major cytoskeletal protein of the red cell membrane. The radiolabel at 89 kDa corresponds closely to the band 3 region which contains the anion transporter of the red cell, while the 76-kDa region overlaps with band 4.2. Finally, the broad peak (43–75 kDa) which contains the greatest amount of [3H]forskolin is identical to the region referred to as zone 4.5 which contains the glucose transport protein in these cells (1, 3). In membranes which were irradiated in the presence of n-glucose, only the latter protein region (i.e. zone 4.5) exhibited any major change in photoincorporation of [3H]forskolin. In this experiment the labeling in this region decreased approximately 45% in the presence of 500 mM D-glucose with virtually no effect on the other polypeptide regions. In other experiments forskolin photolabeling of this region was decreased by up to 60% by...
Photolabeling of the d-Glucose Transporter with [3H]Forskolin

A similar experiment is presented in Fig. 2. In this case membranes were photolysed with [3H]forskolin in the presence of either unlabeled cytochalasin B or cytochalasin E (10 μM). Under these conditions cytochalasin B abolished the labeling in the zone 4.5 region with little effect on the other [3H]forskolin-labeled peaks. Cytochalasin E had no effect on the forskolin-labeling pattern when compared with untreated control gels (data not shown).

Labeling of Stripped Ghost Membranes—When erythrocyte membranes are exposed to low ionic strength alkaline medium in the presence of EDTA, as much as 50% of the membrane protein may be removed (32). The proteins remaining are generally integral membrane proteins which apparently form strong hydrophobic interactions with the lipid bilayer. Under the conditions described above, bands 1, 2, 4.1, 4.2, 5, and 6 are removed, while the remaining membrane protein consists primarily of bands 3, 4.5, and 7 (27). A comparison of the [3H]forskolin-photolabeling pattern between untreated ghost membranes and “stripped” membranes (i.e. extracted with low ionic strength/alkaline EDTA buffer) is shown in Fig. 3. In the untreated membranes (Fig. 3A), the pattern was similar to that seen in Fig. 1 while in the stripped membranes some notable differences were observed (Fig. 3B). In stripped membranes the peak in the region of spectrin was markedly reduced. Since spectrin is almost entirely extracted by this procedure, the results suggest that spectrin is indeed the protein in this region which is labeled. The other two major peaks in these extracted membranes were enriched relative to nonspecific background labeling and correspond to the 89-kDa and 43- to 75-kDa regions. In the untreated membranes (Fig. 3A), the peak between 92 and 68 kDa (i.e. approximately 76 kDa) corresponds closely to the migration of band 4.2 in these gels. Both band 4.2 and the radiolabel in this region disappear following the extraction procedure.

In the experiment depicted in Fig. 4, stripped membranes were photolabeled with either [3H]forskolin or [3H]cytochalasin B and analyzed by sodium dodecyl sulfate-gel electrophoresis. In this experiment the photoincorporation of [3H]cytochalasin B into the glucose transporter was approximately 10-fold greater on a mol basis than [3H]forskolin incorporation into the same region. However, both patterns of incorporation were identical with respect to the electrophoretic mobility of the glucose transporter proteins.

Treatment of Intact Labeled Membranes with Glycosidase—It has previously been demonstrated that treatment of the glucose transporter with endo-β-galactosidase results in removal of around 70% of the carbohydrate from this glycopro-
tein and results in a sharpening of the electrophoretic profile as well as increased mobility (33). We photolabeled intact ghost membranes with either [3H]forskolin or [3H]cytochalasin B and then incubated the membranes with endo-β-galactosidase at 37 °C for 60 min. The results are shown in Fig. 5. Treatment of the [3H]cytochalasin B-labeled membranes (Fig. 5A) resulted in a dramatic sharpening of the peak profile as well as a shift of the labeled region from 54 kDa (peak center) to 44 kDa. In forskolin-treated membranes (Fig. 5B), an identical shift was observed for the [3H]forskolin-labeled transporter with no change in either the high molecular mass peak or in the 92-kDa peak. The marked decrease in the peak associated with the band 42 kDa observed in this figure may be due to a shift of overlapping glucose transporter protein to the lower region of the gel or may mean that this protein contains glycosidase-sensitive carbohydrate.

Membrane Trypsinization—An additional comparison was made between stripped membrane proteins which were forskolin labeled, or cytochalasin B labeled and then trypsinized in situ following photoincorporation. In Fig. 6A the profile for incorporation of [3H]forskolin into erythrocyte membrane proteins is shown. After the membranes were photolabeled with forskolin they were then subjected to treatment with trypsin (Fig. 6B). This treatment was sufficient to cause complete disappearance of bands 3 and 4.5 as judged by Coomassie Blue staining (data not shown). This was accompanied by a complete loss of radiolabel from these regions of labeling in the controls. Only one peak was detected following this treatment, a sharply defined peak corresponding to 17 kDa. When the same membranes were labeled with [3H]cytochalasin B instead of forskolin and then trypsinized (Fig. 6C), a single sharp peak also at 17 kDa which has been shown to be a fragment of the glucose transporter (5-8) was observed. Both peaks are superimposable and suggest that the forskolin-labeled peak is the same trypsin fragment of the glucose transporter as that obtained from cytochalasin B-labeled membranes.

Photolabeling Inhibition Studies—In view of the total inhibition of [3H]forskolin photoincorporation into the glucose transport proteins by cytochalasin B (Fig. 4), experiments were performed to determine if forskolin would inhibit cytochalasin B photolabeling of the transporter. In these experiments membranes were photolabeled with [3H]cytochalasin B in the presence of increasing concentrations of unlabeled forskolin (Fig. 7). As the forskolin concentration increased from 0 to 50 μM, the photoincorporation of [3H]cytochalasin B decreased dramatically to only 20% of control values.
Photolabeling of the d-Glucose Transporter with [3H]Forskolin

In a similar type of experiment the photoincorporation of [3H]forskolin was examined in the presence of increasing concentrations of d-glucose (Fig. 8). In this experiment t-glucose had no effect on the labeling of any of the [3H] forskolin peaks while d-glucose affected only incorporation of label into the 43- to 75-kDa glucose transporter region (results similar to Fig. 1). In Fig. 8 the percent inhibition of [3H]forskolin incorporation into the transporter region is plotted as a function of increasing concentrations of d-glucose. The approximate half-maximal inhibition of glucose-sensitive photolabeling in this experiment corresponds to approximately 70 mM d-glucose.

DISCUSSION

In addition to stimulating adenylate cyclase, forskolin is known to be a potent inhibitor of glucose transport in a number of cells including the erythrocyte (23-25). Recent studies, however, have suggested that the inhibition of transport is independent of elevated cAMP levels (23-26). In particular, one recent investigation (24) provides strong evidence that this inhibitory effect on sugar transport occurs via a direct interaction with the transport system itself.

The results of the present study indicate that [3H]forskolin indeed binds directly to the glucose transporter and can be used as a photoaffinity label for this system in the human red blood cell. A number of experimental results points to this conclusion. First of all a region of proteins, 43-75 kDa, which is photolabeled by [3H]forskolin exhibits the same pattern of labeling and occurs in the same region as those proteins (zone 4.5) previously identified as the glucose transport system. Our results also indicate that the forskolin photolabeling of these proteins in zone 4.5 is strongly inhibited by d- but not t-glucose in a concentration-dependent manner. The pattern of labeling in this region is identical to that of [3H]cytochalasin B photolabeling, and our study suggests that these two compounds may have similar sites of interaction. Thus, unlabeled forskolin is capable of blocking photoincorporation of [3H]cytochalasin B into the transporter proteins in a concentration-dependent manner. This result agrees with a previous study which demonstrated that forskolin inhibits d-glucose-sensitive, reversible cytochalasin B binding (24). In addition we have shown that unlabeled cytochalasin B inhibits photoincorporation of [3H]forskolin into the transporter region while cytochalasin E, which does not block transport, had no effect. Additional evidence for the labeling of glucose transporter proteins by forskolin is provided by the observation involving enzymatic treatment of the labeled membranes. Previous studies have shown that trypsinization of either erythrocyte membranes or purified, reconstituted glucose transport proteins results in the generation of a fragment of 18-21 kDa which contains the cytochalasin B binding site located near the cytoplasmic domain (5, 7, 8), as well as an external glucose-sensitive site (10). In the present study treatment of protein-depleted, forskolin-labeled membranes results in the generation of a 17-kDa fragment which migrates identically with the cytochalasin B-labeled fragment on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels.

In addition, treatment of intact membranes with endo-β-galactosidase results in a sharpening of the 43- to 75-kDa forskolin-labeled band to a peak of approximately 44 kDa, which again is identical to the pattern obtained with the same treatment on cytochalasin B-labeled membranes and is consistent with the evidence that the transporters are heterogeneously glycosylated proteins (8, 39). Finally, membranes which had been partially stripped of extrinsic proteins in the presence of low ionic strength EDTA-containing buffer also exhibited a characteristic enrichment of transporter proteins which was also reflected in an increase in the specific activity (i.e., dpm/mg of total membrane protein) of [3H]forskolin photoincorporation.

The concentration of forskolin previously reported to achieve a half-maximal inhibition of 3-0-methylglucose transport in the red cell is 75 μM (24). In this study the concentration of forskolin which produced a half-maximal inhibition of cytochalasin B photoincorporation into band 4.5 was approximately 9.3 μM, while a concentration of approximately 70 mM d-glucose was required for half-maximal inhibition of photoincorporation of [3H]forskolin into the transporter. The actual affinity constant for forskolin binding to the glucose transporter has not been determined. However, based on the relative photoincorporation interactions with cytochalasin B and d-glucose described in this report, it would appear that forskolin has an affinity for the transporter which is approximately an order of magnitude lower than that of cytochalasin B. Cytochalasin B has been reported to inhibit glucose transport with a Ks of 2.6 - 4 x 10^{-7} M and an apparent binding dissociation constant (Kd) of 3-5 x 10^{-7} M (34). Furthermore, in studies on cytochalasin B photolabeling of the glucose
transporter the half-maximal concentration for incorporation was 0.6-2 μM (12, 14). Detailed kinetic analysis of binding would have to be performed in order to ascertain if these differences between cytochalasin B and forskolin are due to a lower affinity of forskolin for the transporter or reflect different efficiencies of photoincorporation.

Of additional interest is the ability of forskolin to photoincorporate into several other regions of red cell membrane protein, all of which are unaffected by the presence of either D-glucose or cytochalasin B. These include a high molecular mass region of approximately 216 kDa as well as 89 and 76 kDa. We believe that the 216-kDa peak corresponds to the lower band of spectrin based on the following evidence. First, when labeled membranes were run on lower percent acrylamide gels in which the two spectrin bands were widely separated, the forskolin-labeled peak mobility coincided exactly with the migration of lower spectrin band (data not shown). Secondly, when membranes were stripped of extrinsic proteins under conditions where the two spectrin bands were barely visible by Coomassie Blue staining, the amount of [3H]forskolin incorporated into this region was significantly reduced (in some cases, virtually to background levels). Thus, the same procedure which extracted spectrin also extracted the forskolin-labeled protein in this region, in contrast to the enrichment of labeling observed in the glucose transporter region following this treatment. Whereas coincident extraction and mobility on one-dimensional gels is far from proof of identity, we feel that the great preponderance of spectrin in the erythrocyte membrane protein composition results in a small amount of low affinity incorporation of forskolin into this band.

Forskolin labeling of the band 3 region is more difficult to interpret. Based on the estimated relative incorporation of forskolin into band 3 compared with the glucose transporter, the binding affinity is much higher for the transporter than for the band 3 labeling. This is a qualitative judgement based on the observation that decreasing concentrations of forskolin resulted in an increased percentage of the total radiolabel appearing in the transporter peak relative to the other regions, including band 3. The identity of the 89-kDa protein labeled by forskolin is unknown, since other proteins besides the erythrocyte anion transporter migrate in this region. Results from this study indicate that the labeled proteins are resistant to low ionic strength/EDTA extraction and insensitive to treatment with endo-β-galactosidase but sensitive to in situ trypsinization.

A third 76 kDa-peak of glucose-insensitive labeling is consistently observed in the same region as band 4. Again, while the actual identity of this protein is unknown, it does possess the characteristics of being extracted with low ionic strength/EDTA buffer and trypsin sensitivity.

The question of the efficacy of this photoprobe as a potential affinity label for adenylate cyclase is not answered by this study. Thus, while forskolin is reported to bind to adenylate cyclase with high affinity (22), the erythrocyte membrane would be a poor choice to attempt to label this enzyme due to the low activity (and presumably low concentration) in this cell. Several recent reports indicate that forskolin activates the catalytic subunit of adenylate cyclase directly and that the molecular mass of this protein in heart and brain tissue is approximately 120-150 kDa (35, 36). We were unable to observe any significant peak of forskolin labeling in red cell proteins in this region of the gel with either intact or stripped membranes. Attempts to label adenylate cyclase in other tissue plasma membranes with this method has thus far been unsuccessful. This is not surprising considering both the low concentrations of adenylate cyclase in most tissue membranes and the low efficiency of photoincorporation with the current method. Experiments are in progress to attempt to utilize this method to label this enzyme in membranes which have higher concentrations as well as labeling more purified enzyme preparations.

The present report demonstrates that forskolin indeed interacts directly with the glucose transporter in human erythrocytes. This confirms what has been suggested previously as a result of kinetic studies on the potent inhibitory effects of forskolin on glucose transport in erythrocytes. We strongly suspect that the inhibitory action of the forskolin on sugar transport observed in other cells such as adipocytes (23, 26) and cardiomyocytes (25) is also a result of forskolin binding directly to the transporter. We currently are attempting to apply this technique to other cell systems. However, we do not believe that this compound is a particularly good photoaffinity labeling agent for the glucose transporter due to its low efficiency of incorporation and high degree of nonspecific labeling. It may be useful as a tool for probing inhibitory sites on the purified transporter, however. In view of our findings, it may be necessary to re-evaluate some of the previous binding data related to forskolin binding to plasma membranes in a number of cells. The glucose transporter is found in the plasma membranes of almost all animal cells. Although the human erythrocyte contains the largest number of these proteins, substantial numbers are found in other cell types (1-3). If these transporters do have a significant affinity for binding forskolin then this binding likely would have an impact on the determination of the binding constants for forskolin-adenylate cyclase interactions. The significance of the observed photolabeling (and putative binding) of the other erythrocyte membrane proteins by forskolin remains to be determined. The present report indicates that forskolin represents a new affinity label for the D-glucose transporter in human erythrocytes which we hope will prove to be a valuable probe in future studies on the structure and function of this protein.

Acknowledgment—We thank Dr. Arnold E. Ruoho for his many helpful discussions and suggestions.

REFERENCES

1. Jones, M. N., and Nickson, J. K. (1981) Biochim. Biophys. Acta 650, 1-20
2. Carruthers, A. (1984) Prog. Biophys. Mol. Biol. 43, 33-59
3. Wheeler, T. J., and Hinkle, P. C. (1985) Annu. Rev. Physiol. 47, 503-517
4. Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H., Allard, J., Lienhard, G., and Lodish, H. (1985) Science 229, 941-945
5. Cairns, M. T., Eliot, D. A., Scudder, P. R., and Baldwin, S. A. (1984) Biochem. J. 221, 179-186
6. Klip, A., Deziel, M., and Walker, D. (1984) Biochem. Biophys. Res. Commun. 122, 218-224
7. Deziel, M. R., and Rothstein, A. (1984) Biochim. Biophys. Acta 776, 10-20
8. Shannahon, M. F., and O’Artell-Ellis, J. (1984) J. Biol. Chem. 259, 13878-13884
9. Ishi, T., Tillotson, L. G., and Isselbacher, K. J. (1985) Biochim. Biophys. Acta 832, 14-21
10. Holman, G. D., Parkar, B. A., and Midgley, P. J. W. (1986) Biochim. Biophys. Acta 856, 115-126
11. Pessin, J. E., Tillotson, L. G., and Isselbacher, K. J. (1985) Biochim. Biophys. Acta 853, 1221-224
12. Carter-Su, C., Pessin, J. E., Mora, R., Gitomer, W., and Czech, M. P. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2286-2289
13. Carter-Su, C., Pessin, J. E., Mora, R., Gitomer, W., and Czech, M. P. (1983) J. Biol. Chem. 257, 5419-5425
14. Shannahon, M. F. (1983) Biochemistry 22, 2750-2756

1 M. F. Shannahon, unpublished observation.
Photolabeling of the D-Glucose Transporter with $[^3H]$Forskolin

15. Shanahan, M. F., Olson, S. A., Weber, M. J., Lienhard, G., and Gorga, J. C. (1982) Biochem. Biophys. Res. Commun. 107, 38-43
16. Johnson, L. W., and Smith, C. H. (1982) Biochem. Biophys. Res. Commun. 109, 408-413
17. Ingermann, R. L., Bissonnette, J. M., and Koch, P. L. (1983) Biochim. Biophys. Acta 730, 57-63
18. Klip, A., Walker, D., Ransome, K. J., Schroer, D. W., and Lienhard, G. E. (1983) Arch. Biochem. Biophys. 226, 198-205
19. Roberts, S. J., Tanner, M. J. A., and Denton, R. M. (1982) Biochem. J. 205, 139-145
20. Weber, T. M., and Eichholz, A. (1985) Biochim. Biophys. Acta 812, 503-511
21. Shanahan, M. F., Wadzinski, B. E., Lowndes, J. M., and Ruoho, A. E. (1985) J. Biol. Chem. 260, 10897-10900
22. Seamon, K. B., and Daley, J. W. (1981) J. Cyclic Nucleotide Res. 7, 201-224
23. Kashiwagi, A., Huecksteadt, T. P., and Foley, J. E. (1983) J. Biol. Chem. 258, 13685-13692
24. Sergeant, S., and Kim, H. D. (1985) J. Biol. Chem. 260, 14677-14682
25. Shanahan, M. F., Edwards, B. M., and Ruoho, A. E. (1986) Biochim. Biophys. Acta 887, 121-129
26. Mills, I., Moreno, F. J., and Fain, J. N. (1984) Endocrinology 115, 1066-1069
27. Steck, T. L., and Kant, J. A. (1974) Methods Enzymol. 31, 172-180
28. Sogin, D. C., and Hinkle, P. C. (1978) J. Supramol. Struct. 8, 447-453
29. Giulian, G. G., Moss, R. L., and Greaser, M. (1983) Anal. Biochem. 129, 227-287
30. Peterson, G. L. (1977) Anal. Biochem. 83, 346-356
31. Deziel, M., Pegg, W., Mack, E., Rothstein, A., and Klip, A. (1984) Biochim. Biophys. Acta 772, 403-406
32. Steck, T. L., and Yu, J. (1973) J. Supramol. Struct. 1, 220-232
33. Gorga, F. R., Baldwin, S. A., and Lienhard, G. E. (1979) Biochem. Biophys. Res. Commun. 91, 955-961
34. Jung, C. Y., and Rampal, A. L. (1977) J. Biol. Chem. 252, 5456-5463
35. Pfeuffer, E., Dreher, R.-M., Metzger, H., and Pfeuffer, T. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 3086-3090
36. Smigel, M. D. (1986) J. Biol. Chem. 261, 1976-1982