ELSEVIER

Biochimica et Biophysica Acta 1265 (1995) 73–78

BBA Biochimica et Biophysica Acta

Insulin action on cardiac glucose transport: studies on the role of protein kinase C

Martina Russ, Jürgen Eckel *

Laboratory of Molecular Cardiology, Diabetes Research Institute, Auf'm Hennekamp 65, D-40225 Düsseldorf, Germany

Received 30 August 1994; accepted 6 October 1994

Abstract

Isolated ventricular cardiomyocytes from adult rat have been used to elucidate a possible relationship between protein kinase C (PKC) and the stimulatory action of insulin on cardiac glucose transport. Cells were incubated in the presence of either insulin or phospholipase C from *Clostridium perfringens* (PLC-Cp) and intracellular *sn*-1,2-diacylglycerol (DAG) levels and initial rates of 3-*O*-methylglucose transport were determined. Insulin had no effect on the DAG mass level, whereas it was elevated by PLC-Cp to 200% of control. Under these conditions the hormone produced a 2.7-fold stimulation of glucose transport with no significant effect of PLC-Cp. Insulin was unable to produce a redistribution of PKC, whereas phorbol 12-myristate 13-acetate (PMA) increased membrane associated PKC twofold. The PKC inhibitors tamoxifen and staurosporine did not interfere with glucose transport stimulation by insulin. Furthermore, cells treated with PMA exhibited unaltered basal and maximally insulin stimulated rates of glucose transport. In contrast, at physiological concentrations of insulin the stimulatory action of the hormone was significantly reduced. We conclude from our data that PKC is not involved in insulin action on cardiac glucose transport. However, activation of this enzyme may lead to a modified insulin sensitivity of the cardiac cell.

Keywords: Glucose transport; Insulin; Protein kinase C; Phorbol ester

1. Introduction

A primary and essential step of insulin action consists in the autoactivation of an intrinsic tyrosine kinase activity located in the beta-subunit of the insulin receptor (for review, see [1]). Subsequent steps of insulin signalling remain obscure and a variety of cellular signal transduction elements like G-proteins [2], tyrosine phosphorylated proteins [3], glycolipids [4], and serine/threonine kinases [5] are assumed to modulate or mediate the ultimate action of the hormone.

A serine/threonine kinase which has gained considerable interest as a putative mediator of insulin action is the Ca^{2+}- and phospholipid-dependent protein kinase C (PKC) [6–11]. Evidence supporting this view is based on the observation that (i) insulin stimulates an increase in DAG [6–8] and a translocation of PKC [8,9] in muscle and adipose tissue, (ii) phorbol esters, which are known activators of PKC, induce translocation of Glut4 [10] and at least partly mimick insulin action on hexose transport [8,11], and (iii) down-regulation of PKC blocks stimulation of glucose uptake by insulin [11]. In addition to possibly mediating insulin action, PKC has also been implicated in termination of the insulin signal, a process, which is thought to result from serine phosphorylation of the insulin receptor β-subunit and a concomitant decrease of tyrosine kinase activity [12,13].

Despite the above mentioned studies, the precise function of PKC in insulin signal transduction remains an unresolved issue. Work performed on different rodent skeletal muscle preparations and a variety of cell lines indeed argues against a role for PKC in insulin signalling [14–18]. Thus, DAG remained unaffected by in vitro [14] and in vivo [15] insulin treatment, insulin was unable to induce PKC translocation [16] or phosphorylation of a cellular substrate protein [17], and PMA had only minimal effects on glucose transport [16,18].

Abbreviations: PDD, 4-alpha-phorbol 12,13-didecanoate; DAG, *sn*-1,2-diacylglycerol; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PLC-Cp, phospholipase C from *Clostridium perfringens*.

* Corresponding author. Fax: +49 211 3382603.

0167-4889/95/$09.50 © 1995 Elsevier Science B.V. All rights reserved
SSDI 0167-4889(94)00200-2

In light of these controversial findings we have now used our preparation of ventricular cardiomyocytes [19,20] in order to reevaluate the functional role of PKC for the regulation of glucose transport in a primary muscle cell. These cells express a high level of Glut4, which is translocated to the plasma membrane in response to insulin and contraction, as recently shown by us [21]. In addition to being a putative mediator of insulin action, PKC has also been implicated in the regulation of cardiac contractility [22] and in the transduction of mechanical stimuli to a hypertrophic response [23]. In the present investigation attempts have been made to correlate the level of DAG with glucose transport activity and to compare the stimulatory action of insulin and PMA on PKC activity and hexose transport in isolated cardiac cells. The data clearly show that elevation of DAG mass and activation of PKC are events unrelated to the stimulation of glucose transport by insulin. It is suggested, however, that PKC is able to modulate the sensitivity of cardiomyocytes towards stimulation by insulin.

2. Materials and methods

2.1. Isolation of ventricular cardiomyocytes

Male Wistar rats weighing 280–320 g were used in all experiments. Ca^{2+}-tolerant ventricular myocytes were isolated by perfusion of the heart with collagenase as previously described by us [19]. The final cell suspension was washed three times with Hepes buffer (composition: NaCl 130 mM, KCl 4.8 mM, KH_2PO_4 1.2 mM, Hepes 25 mM, glucose 5 mM, bovine serum albumin 20 g/l (pH 7.4), equilibrated with oxygen) and incubated in silicone-treated Erlenmeyer flasks in a rotating waterbath shaker at 37°C. After 20 min $CaCl_2$ and $MgSO_4$ (final concentration 1 mM) were added and incubation was continued until further use. Cell viability was checked by determination of the percentage of rod-shaped cells and averaged 90–95% under all incubation conditions.

2.2. Measurement of DAG mass

Cardiomyocytes were incubated at 37°C in Hepes buffer for the indicated times. The lipid fraction was then extracted by a modification of the method of Bligh and Dyer [24]. Assay of DAG mass was performed using the Amersham DAG assay reagent system, which is based on the method of Preiss et al. [25]. The DAG in the lipid extract was converted quantitatively to [^{32}P]phosphatidic acid by DAG kinase in the presence of [γ-^{32}P]ATP. [^{32}P]Phosphatidic acid was then separated by polar extraction using silica Amprep minicolumns and the associated radioactivity was determined. DAG levels were calculated from standard curves generated in parallel. Significance of reported differences was evaluated using the null hypothesis and t statistics for paired data.

2.3. 3-O-Methylglucose transport

Transport experiments were performed at 37°C in Hepes buffer containing $MgCl_2$ (1 mM) and $CaCl_2$ (1 mM). The reaction was started by pipetting a 50 μl aliquot of the cell suspension to 50 μl of Hepes buffer containing 3-*O*-[^{14}C]methyl-D-glucose (final concentration 100 μM). Carrier-mediated glucose transport was then determined using a 10 s assay period and L-[^{14}C]glucose in order to correct for simple diffusion as described in earlier reports from this laboratory [19–21].

2.4. Protein kinase C assay

Cardiomyocytes ($5 \cdot 10^5$ cells/0.5 ml) were exposed to PMA or insulin for the indicated times. Cells were then homogenized in a buffer containing 20 mM Hepes, 5 mM EGTA, 0.25 mM sucrose, 5 mM DTT, 1 g/l BSA and 50 μg/ml leupeptin by sonication (five times for 10 s) followed by 9×4 strokes in a glass potter homogenizer. The whole homogenate was centrifuged for 45 min at 100 000 $\times g$ and the supernatant was considered as the cytosolic fraction. The pellet was solubilized in homogenizing buffer containing 0.4% Triton X-100 and centrifuged at 10 000 $\times g$ for 10 min. Both fractions were then applied to DEAE-cellulose columns, which were washed with 2.5 ml of a buffer containing 20 mM Tris-HCl (pH 7.4), 2 mM EDTA, 5 mM EGTA and 2 mM PMSF. PKC was eluted with the same buffer containing 100 mM NaCl. PKC activity was then determined by measuring the Ca^{2+} and phospholipid-dependent transfer of ^{32}P from [^{32}P]ATP to histone III-S. 100 μl of the eluate was added to 150 μl of a solution containing 20 mM Tris-HCl (pH 7.5), 10 mM magnesium acetate, 25 μg histone III-S either in the absence or presence of 0.75 mM $CaCl_2$, 12 μg of phosphatidylserine and 1.6 μg of 1,2-diolein. The reaction was started by addition of 50 μM ATP containing 5 μCi of [^{32}P]ATP per assay and conducted for 30 min at room temperature. Histone was then precipitated by addition of ice-cold 25% trichloroacetic acid and separated by filtration on 0.45 μm nitrocellulose filters. Filters were then counted by liquid scintillation. All data were corrected for phospholipid-independent kinase activity. Translocation of PKC was determined by measuring the ratio of membrane associated activity/total activity (total activity is cytosolic activity plus particulate activity).

2.5. Chemicals

[γ-^{32}P]ATP (6000 Ci/mmol) was purchased from New England Nuclear (Germany). 3-*O*-[^{14}C]Methyl-D-glucose (57 Ci/mmol), L-[1-^{14}C]glucose (58 Ci/mmol), the DAG assay reagent system, and Amprep minicolumns were from

Amersham (Germany). PMA, 4-α-phorbol 12,13-didecanoate (PDD), phosphatidylserine, 1,2-diolein, histone III-S and phospholipase C from *Clostridium perfringens* were obtained from Sigma (Germany). Staurosporine was from Calbiochem (Germany). All other chemicals were of the highest grade commercially available.

3. Results

Intracellular DAG is known to act as the endogenous activator of PKC. Furthermore, it has been suggested that DAG may mediate insulin action directly through an increase in the recruitment and/or intrinsic activity of glucose transporters [7]. As shown in Table 1, insulin was unable to increase the intracellular DAG mass in ventricular cardiomyocytes after 30 min of incubation, whereas under these conditions a large stimulation of glucose transport could be observed. Treatment of cells with PLC-Cp was found to increase the DAG level by a factor of two. However, despite this increase in DAG mass, PLC-Cp did not modify the initial rates of 3-*O*-methylglucose transport (Table 1), in contrast to BC3H-1 myocytes, where both PLC-Cp and exogenously added DAG produced a 2–3-fold increase of glucose transport [7]. In order to rule out a transient increase of DAG, the kinetics of insulin action on DAG mass in cardiomyocytes were investigated. Using a maximally active dose of the hormone, a slight (maximum 19% at 20 min) but insignificant increase was observed at all time points studied (Fig. 1). Similar results were obtained at lower insulin concentrations.

It may be argued that the insulin-dependent DAG pool in cardiomyocytes represents only a small part of total DAG mass or that PKC activation by insulin is mediated by factors other than DAG [8]. We have therefore determined the time-dependent effect of insulin on the membrane association of PKC and compared it to the action of the phorbolester PMA, which was found to induce a rapid translocation of PKC in adult cardiomyocytes [26] including all isoforms present in these cells (α, δ, ϵ), as shown in a very recent report by Pucéat et al. [27]. The membrane association of PKC expressed as the ratio of membrane activity/total activity is presented in Fig. 2. The data clearly show that insulin is unable to induce a translocation of PKC both after a 5 or a 20 min incubation time with the hormone, whereas the phorbol ester increased membrane-associated PKC twofold. It should be noted that the total PKC activity (53.8 pmol/min/10^6 cells) even slightly

Table 1
Effect of insulin and phospholipase C on 1,2-diacylglycerol mass and glucose transport in ventricular cardiomyocytes

Incubation condition	DAG content (nmol/$1.5 \cdot 10^6$ cells)	3-*O*-Methylglucose transport (pmol/10^6 cells × 10 s)
Basal	17.5 ± 2.1 (5)	357 ± 34 (4)
Insulin	14.5 ± 1.4 [a] (5)	945 ± 51 (4)
PLC-Cp	35.3 ± 3.9 (3)	409 ± 77 [a] (4)

Cardiomyocytes were incubated at 37°C for 30 min in the absence or presence of either insulin ($3.5 \cdot 10^{-7}$ M) or PLC-Cp (0.2 U/ml). DAG mass levels and 3-*O*-methylglucose transport were then determined as described in Section 2. Data are mean values ± S.E. for the number of experiments indicated in parentheses.
[a] Not significantly different from basal ($P > 0.05$).

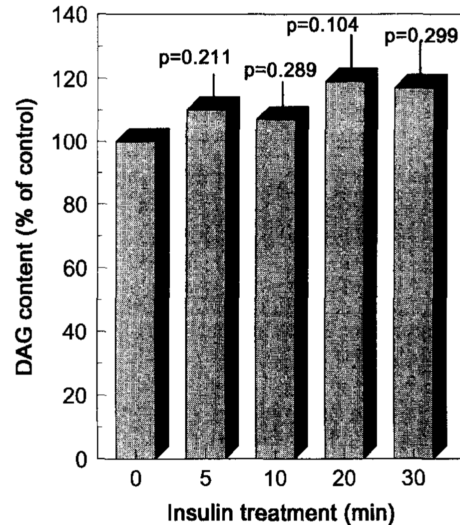

Fig. 1. Time-related effects of insulin on 1,2-diacylglycerol content of ventricular cardiomyocytes. Cells were incubated at 37°C for the indicated times in the absence or presence of insulin ($3.5 \cdot 10^{-7}$ M). The lipid fraction was extracted and DAG mass levels were assayed as described in Section 2. Data are mean values ± S.E. ($n = 3$) expressed as percent of untreated controls. *P* values were determined by paired *t*-test.

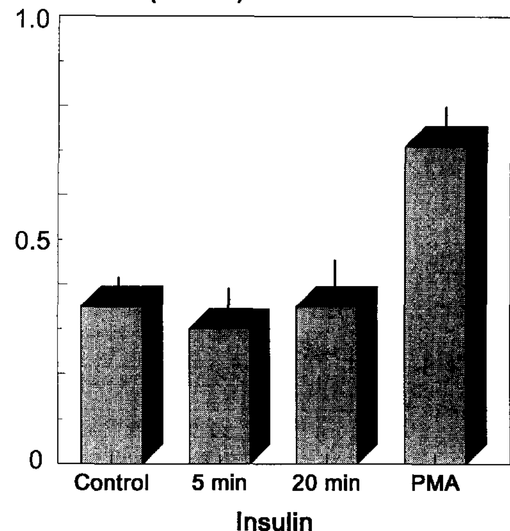

Fig. 2. Effect of insulin and PMA on membrane association of PKC. Cardiomyocytes were incubated in the absence or presence of either insulin ($3.5 \cdot 10^{-7}$ M) or PMA (1 μM, 30 min). Analysis of membrane and cytosolic PKC activity was performed as described in Section 2. PKC distribution is expressed as the ratio of membrane activity (M)/cytosolic activity (C) + M. Data are mean values ± S.E. ($n = 3$–4).

Table 2
Effect of PKC inhibitors on basal and insulin-stimulated glucose transport in cardiomyocytes

Addition	3-*O*-Methylglucose transport (pmol/10^6 cells × 10 s)		
	Basal	Insulin	Increment
Control	485 ± 5	1350 ± 83	865
Tamoxifen	374 ± 24 [a]	1105 ± 79	731
Control	394 ± 37	1126 ± 35	732
Staurosporine	162 ± 35 [a]	804 ± 31	642

Cells were incubated for 30 min in the absence (control) or presence of tamoxifen (100 μM) or staurosporine (1 μM), then stimulated for 30 min with insulin ($3.5 \cdot 10^{-7}$ M) prior to assay of initial 3-*O*-methylglucose transport rates. Data are mean values ± S.E. of 3–5 separate experiments.
[a] Significantly different from control with $P < 0.05$.

(30%) decreased in insulin-treated cells, whereas it remained unaffected after PMA treatment.

The data reported so far strongly suggest that insulin action on cardiac glucose transport is not mediated by PKC. Additional evidence for this conclusion was obtained from experiments using the protein kinase C inhibitors tamoxifen and staurosporine. Both drugs were found to reduce the basal glucose transport rates by 20–60%. However, the incremental increase in glucose transport due to stimulation with insulin was not significantly affected by the PKC inhibitors (Table 2).

Despite apparently not being involved in the transduction of the insulin signal to the cardiac glucose transporter, PKC may interfere with insulin signalling potentially leading to a loss of insulin sensitivity or responsiveness, as shown in other cellular systems [12,13,28]. We have therefore evaluated the effect of PKC activation by PMA on basal and insulin-stimulated glucose transport. As shown

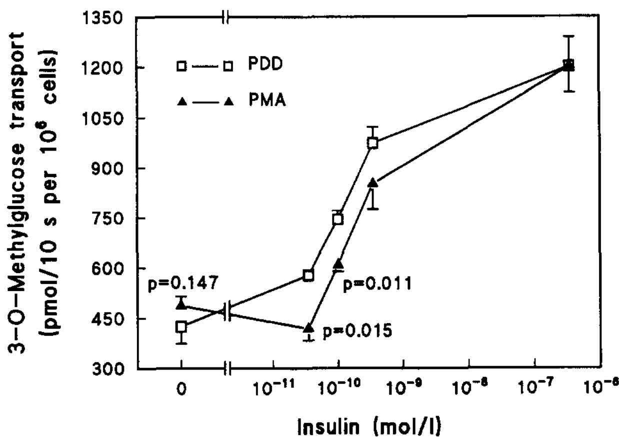

Fig. 3. Dose–response relationship for insulin stimulated transport of 3-*O*-methylglucose. Myocytes were treated for 30 min with 1 μM of either PDD or PMA. Incubation was then continued for 60 min in the presence of increasing concentrations of insulin ($3.5 \cdot 10^{-11}$ to $3.5 \cdot 10^{-7}$ mol/l). Hexose uptake was determined as outlined in Fig. 1. The data shown are mean values ± S.E. ($n = 3$–6). *P* values were determined for PDD versus PMA at corresponding insulin concentrations using paired *t*-test.

in Fig. 3, PMA produced no significant increase of basal glucose transport when compared to the inactive phorbol analogue PDD. Furthermore, the maximal response was also not affected by PKC activation. However, PMA was found to produce a rightward shift of the dose–response curve resulting in significantly lower insulin-stimulated transport rates at $3 \cdot 10^{-11}$ and 10^{-10} M (Fig. 3), suggesting a reduced insulin sensitivity of PMA-treated cardiac cells. Under these conditions a slight (21%) but significant ($P = 0.0065$) reduction of insulin binding could also be detected.

4. Discussion

The putative involvement of PKC in the process of signal transduction from the insulin receptor to the glucose transporter has remained a controversial and unresolved issue. The present investigation has examined this relationship in ventricular cardiomyocytes, since (a) these primary muscle cells express a high level of the insulin responsive glucose transporter Glut4 [21], and (b) PKC is of additional importance for the regulation of contractility [22] and gene expression [23] in these cells. Our data suggest that the acute action of insulin on cardiac glucose uptake is independent of PKC activation. Three lines of evidence support this conclusion. First, insulin was unable to modify the DAG content of cardiomyocytes, second, no activation/translocation of PKC in response to the hormone could be detected, and third, inhibitors of PKC did not affect the insulin-stimulated glucose uptake.

A major finding of the present report consists in the observation that insulin was unable to increase the DAG mass in cardiomyocytes. This contrasts with the BC3H-1 myocytes, which show a rapid (2 min) and sustained 2–3-fold increase of DAG-content in response to insulin resulting from multiple sources [6,7,29]. However, this large response may be specific for the BC3H-1 cell, since Farese's group reported a relatively small DAG increase (20–30%) in skeletal muscle after in vitro [30] and in vivo [8] insulin treatment. Furthermore, Turinsky et al. [15] observed an unaltered DAG level in rat skeletal muscle after in vivo administration of insulin and Merrall et al. [14] reported the inability of insulin to modify the DAG level of 3T3-L1 fibroblasts. Interestingly, phospholipase C had essentially no effect on glucose uptake by cardiomyocytes despite inducing a twofold increase in DAG mass in these cells. On the other hand, PLC-Cp was found to mimick insulin action in BC3H-1 myocytes [7] and adipocytes [31]. However, it has to be considered that PLC-Cp is also able to increase the cytosolic Ca^{2+} concentration which may then lead to an increased glucose transport, as shown by Henriksen et al. [16] in skeletal muscle. We therefore conclude that lipolytically produced DAG does not act as a mediator of insulin action on cardiac glucose transport.

As outlined by Farese's group [8], a small but metabolically active, insulin-dependent DAG pool or factors other than DAG could participate in the PKC translocation response. However, we have ruled out this possibility for the cardiomyocytes by (I) showing that insulin is unable to promote the membrane association of PKC, and (II) demonstrating an unaltered insulin action in the presence of PKC inhibitors. In agreement with our findings, no effect of insulin on PKC translocation or activation was observed in L6 skeletal muscle cells [32] and 3T3-L1 fibroblasts [14], it was however assayed in rat adipocytes [9]. In rat skeletal muscle an insulin-induced translocation of PKC was reported for soleus and gastrocnemius muscle [8], but could not be detected in epitrochlearis muscles [16]. A possible key to explaining these apparent controversial findings obtained by different laboratories may be related to a different pattern of PKC isoforms in different tissues or cell lines, since insulin and phorbol esters were found to have overlapping and distinctly different effects on the redistribution of PKC isoforms [33]. Thus, insulin but not PMA stimulated the translocation of PKC-β in adipocytes, whereas PMA was much more effective on the redistribution of PKC-α [33]. Very recently, Pucéat et al. showed that PKC-β is not expressed in adult rat cardiomyocytes [27]. It may be speculated that the lack of insulin action on PKC translocation shown in the present study is related to the specific pattern of PKC isoform expression in the heart. This hypothesis needs further investigation. Nevertheless, the data clearly indicate that PKC translocation/activation is not an essential requirement for insulin action on muscle tissue.

Additional evidence to exclude the involvement of PKC signalling to cardiomyocyte glucose transport was obtained from the inability of PMA to affect hexose transport in these cells. Consistently, only minimal effects of PMA on glucose transport in different rat skeletal muscle preparations have been reported [16,18]. In contrast to our findings, van de Werve et al. [34] observed a 3-fold increase in 3-*O*-methylglucose efflux by PMA in isolated perfused hearts, suggesting stimulation of glucose transport by PKC activation. However, the mechanisms of PMA-stimulated glucose transport remain controversial. Thus, Gibbs et al. [35] recently showed that insulin and PMA act via different mechanisms, whereas earlier work suggested that PMA induces a translocation of glucose transporters to an extent comparable with that induced by insulin [36]. Furthermore, PMA acts on both Glut1 and Glut4 [35] and this may at least partly explain the different results obtained with cardiomyocytes and perfused heart tissue [34].

Despite being unable to affect basal glucose transport, PMA was found to produce a rightward shift of the dose–response curve for insulin-stimulated glucose transport resulting in a reduced sensitivity at physiological concentrations of the hormone. Thus, PKC activation is able to modify insulin signalling in the cardiac cell. This finding agrees well with the data obtained in perfused heart tissue [34] and earlier observations on isolated adipocytes [12]. In these cells the insulin resistance induced by PMA was shown to result from a large inhibition of the insulin receptor kinase, most probably due to serine phosphorylation of the insulin receptor [12]. The precise location of PMA-induced desensitization in cardiomyocytes remains to be elucidated. Reduced receptor binding, as reported here, may only marginally contribute to this process and additional alterations at the receptor [12] and the postreceptor level [28] have to be considered.

Taken together, the present study shows that the DAG/PKC signalling system does not mediate the stimulatory action of insulin on cardiac glucose transport. Instead, PKC activation induces insulin resistance by modifying the sensitivity of the cardiac cell.

Acknowledgements

The authors thank Ulrike Kutscher for performing the DAG determinations. This work was supported by the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen, the Bundesministerium für Gesundheit, and the Deutsche Forschungsgemeinschaft (SFB 351, C 2).

References

[1] Häring, H.U. (1991) Diabetologia 34, 848–861.
[2] Russ, M., Reinauer, H. and Eckel, J. (1992) FEBS Lett. 314, 72–76.
[3] White, M., Maron, R. and Kahn, C.R. (1985) Nature 163, 76–80.
[4] Mato, J.M. (1989) Cell. Signal. 1, 143–146.
[5] Czech, M.P., Klarlund, J.K., Yagaloff, K.A., Bradford, A.P. and Lewis, R.E. (1986) J. Biol. Chem. 263, 11017–11020.
[6] Farese, R.V., Konda, T.S., Davis, J.S., Standaert, M.L., Pollet, R.J. and Cooper, D.R. (1987) Science 236, 586–589.
[7] Standaert, M.L., Farese, R.V., Cooper, D.R. and Pollet, R.J. (1988) J. Biol. Chem. 263, 8696–8706.
[8] Yu, B., Standaert, M.L., Arnold, T., Hernandez, H., Watson, J., Ways, K., Cooper, D.R. and Farese, R.V. (1992) Endocrinology 130, 3345–3355.
[9] Ishizuka, T., Cooper, D.R. and Farese, R.V. (1989) FEBS Lett. 257, 337–340.
[10] Vogt, B., Mushack, J., Seffer, E. and Häring, H.U. (1991) Biochem. J. 275, 597–600.
[11] Tanti, J.-F., Rochet, N., Gremeaux, T., van Obberghen, E. and Le Marchand-Brustel, Y. (1989) Biochem. J. 258, 141–146.
[12] Häring, H., Kirsch, D., Obermaier, B., Ermel, B. and Machicao, F. (1986) J. Biol. Chem. 261, 3869–3875.
[13] Takayama, S., White, M.F. and Kahn, C.R. (1988) J. Biol. Chem. 263, 3340–3347.
[14] Merrall, N.W., Wakelam, M.J.O., Plevin, R. and Gould, G.W. (1993) Biochim. Biophys. Acta 1177, 191–198.
[15] Turinsky, J., Bayly, B.P. and O'Sullivan, D.M. (1990) J. Biol. Chem. 265, 7933–7938.
[16] Henriksen, E.J., Rodnick, K.J. and Holloszy, J.O. (1989) J. Biol. Chem. 264, 21536–21543.
[17] Blackshear, P.J., McNeill Haupt, D. and Stumpo, D.J. (1991) J. Biol. Chem. 266, 10946–10952.

[18] Sowell, M.O., Treutelaar, M.K., Burant, C.F. and Buse, M.G. (1988) Diabetes 37, 499–506.
[19] Eckel, J., Pandalis, G. and Reinauer, H. (1983) Biochem. J. 212, 385–392.
[20] Eckel, J., Gerlach-Eskuchen, E. and Reinauer, H. (1990) Biochem. J. 272, 691–696.
[21] Kolter, T., Uphues, I., Wichelhaus, A., Reinauer, H. and Eckel, J. (1992) Biochem. Biophys. Res. Commun. 189, 1207–1214.
[22] Krämer, B.K., Smith, T.W. and Kelly, R.A. (1991) Circ. Res. 68, 269–279.
[23] Komuro, I., Katoh, Y., Kaida, T., Shibazaki, Y., Kurabayashi, M., Hoh, E., Takaku, F. and Yazaki, Y. (1991) J. Biol. Chem. 266, 1265–1268.
[24] Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[25] Preiss, J., Loomis, C.R., Bishops, W.R., Stein, R., Niedel, J.E. and Bell, R.M. (1986) J. Biol. Chem. 261, 8597–8600.
[26] Capogrossi, M.C., Kaku, T., Filburn, C.R., Pelto, D.J., Hansford, R.G., Spurgeon, H.A. and Lakatta, E.G. (1990) Circ. Res. 66, 1143–1155.
[27] Pucéat, M., Hilal-Dandan, R., Strulovici, B., Brunton, L.L. and Brown, J.H. (1994) J. Biol. Chem. 269, 16938–16944.
[28] Chin, J.E., Dickens, M., Tavare, J.M. and Roth, R.A. (1993) J. Biol. Chem. 268, 6338–6347.
[29] Farese, R.V., Cooper, D.R., Konda, T.S., Nair, G., Standaert, M.L., Davis, J.S. and Pollet, R.J. (1988) Biochem. J. 256, 175–184.
[30] Ishizuka, T., Cooper, D.R., Hernandez, H., Buckley, D., Standaert, M.L. and Farese, R.V. (1990) Diabetes 39, 181–190.
[31] Christensen, R.L., Shade, D.L., Graves, C.B. and McDonald, J. (1987) Int. J. Biochem. 19, 259–265.
[32] Klip, A. and Ramlal, T. (1987) Biochem. J. 242, 131–136.
[33] Farese, R.V., Standaert, M.L., Francois, A.J., Ways, K., Arnold, T.P., Hernandez, H. and Cooper, D.R. (1992) Biochem. J. 288, 319–323.
[34] Van de Werve, G., Zaninetti, D., Lang, U., Valloton, M.B. and Jeanrenaud, B. (1987) Diabetes 36, 310–314.
[35] Gibbs, E.M., Calderhead, D.M., Holman, G.D. and Gould, G.W. (1991) Biochem. J. 275, 145–150.
[36] Mühlbacher, C., Karnieli, E., Schaff, P., Obermaier, B., Mushack, J., Rattenhuber, E. and Häring, H.U. (1988) Biochem. J. 249, 865–870.