Regulation of GLUT4 Gene Expression by Arachidonic Acid

EVIDENCE FOR MULTIPLE PATHWAYS, ONE OF WHICH REQUIRES OXIDATION TO PROSTAGLANDIN E₂*

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Sheree D. Long and Phillip H. Pekala‡

From the Department of Biochemistry, School of Medicine, East Carolina University, Greenville, North Carolina 27858

We have previously described the ability of arachidonic acid (AA) to regulate GLUT4 gene expression (Tebbey, P. W., McGowan, K. M., Stephens, J. M., Buttte, T. M., and Pekala, P. H. (1994) J. Biol. Chem. 269, 639-644). Chronic exposure (48 h) of fully differentiated 3T3-L1 cells to AA resulted in an ~90% suppression of GLUT4 mRNA accumulation. This decrease was demonstrated to be due to a 50% decrease in GLUT4 gene transcription as well as a destabilization of the GLUT4 message (t₁/₂, decreased from 8.0 to 4.6 h). In the current study we have identified, at least in part, the mechanism by which AA exerts its effects on GLUT4 expression. Compatible with a cyclooxygenase mediated event, the AA-induced suppression of GLUT4 mRNA was abolished by pretreating the cells with the inhibitor, indomethacin. Consistent with this observation, exposure of the cells to 10 μM PGE₂ mimicked the effect of AA, in contrast to products of the lipoxygenase pathway which were unable to suppress GLUT4 mRNA content. Quantification of the conversion of AA to PGE₂ demonstrated a 50-fold increase in PGE₂ released into the media within 7 h of AA addition. Cyclic AMP levels were also increased 50-fold with AA treatment consistent with PGE₂ activation of adenylate cyclase. Various long chain fatty acids, including the nonmetabolizable analog of AA, eicosatetraenoic acid (ETYA), also decreased GLUT4 mRNA levels. The effect of ETYA, a potent inhibitor of both lipo- and cyclooxygenases and a potent activator of peroxisome proliferator activated receptors (PPARs), suggested the presence of a second pathway where nonmetabolized fatty acid functioned to suppress GLUT4 mRNA levels. Further support for a PPAR-mediated mechanism was obtained by exposure of the cells to the classic PPAR activator, clofibrate, which resulted in a ~75% decrease in GLUT4 mRNA content. Nuclear extracts prepared from the adipocytes contained a protein complex that bound to the PPAR responsive element (PPRE) found in the promoter of the fatty acyl-CoA oxidase gene. When the adipocytes were treated with either AA or ETYA, binding to the PPRE was disrupted, consistent with an ability of these fatty acids to control gene expression by altering the occupation of a PPRE. However, a perfect PPRE has yet to be identified in the GLUT4 promoter, but this does not rule the possibility of a PPAR playing an indirect role in the AA-induced GLUT4 mRNA suppression.

Glucose transport across the plasma membrane represents the rate-limiting step in glucose metabolism and is a highly regulated process in the animal cell. Facilitated diffusion of glucose into the cell is carried out by a family of tissue-specific transport proteins known as the glucose transporters (GLUT1 through GLUT5 and GLUT7). These integral membrane proteins are members of a gene family in which tissue-specific expression of one or more members will in part determine the net rate of glucose entry into the cell. In addition to tissue-specific expression, hormones, growth factors, and fatty acids can influence the net flux of glucose across the plasma membrane (Kahn, 1992; James et al., 1989; Birnbaum, 1989; Cornelius et al., 1990; Stephens and Pekala, 1992; Tebbey et al., 1994). In addition to roles in the biosynthesis of lipids and energy production, fatty acids are involved in the regulation of glucose metabolism. Fatty acids in general and arachidonic acid (AA) specifically have been demonstrated to be physiological regulators of the adipocyte glucose transport system (Hardy et al., 1991; Murer et al., 1992; Hunnicut et al., 1994; Tebbey et al., 1994). Arachidonic acid (20:4), a major metabolically important polyunsaturated fatty acid present in mammalian cells, is synthesized in the liver from dietary linoleic acid (18:2) and then transported via serum albumin or lipoproteins to various tissues. Serum levels of AA are low relative to other fatty acids except in obesity and diabetes where levels can be significantly elevated over normal matched controls (Distel et al., 1992; Svedberg et al., 1990; Grunfeld et al., 1981). In addition, many cells possess a high affinity arachidonyl-CoA synthetase which facilitates selective accumulation of AA even when other fatty acid species are in excess (Neufeld et al., 1983; Taylor et al., 1985). Such characteristics imply an important role for AA in cellular growth and function and as evidence for such, AA has been implicated in the regulation of gene expression (Tebbey and Buttte, 1992; Glaslow et al., 1992; Ntambi, 1992; Clarke and Abraham, 1992; Tebbey et al., 1994). Specifically, AA was found to suppress the transcription rate of a number of genes, including: the stearoyl-CoA desaturase 2 in T-cells (Tebbey and Buttte, 1992), the hepatic fatty acid synthase (Clarke and Abraham, 1992), and GLUT4 in 3T3-L1 adipocytes (Tebbey et al., 1994). Interestingly, stearoyl-CoA desaturase 2 and GLUT4 are coordinately expressed during the differentiation process in the 3T3-L1 adipocytes (Kaestner et al., 1989, 1990) and results obtained in transient co-transfection assays suggest the possibility of identical mechanisms of activation of the promoters for AA metabolism.
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both the GLUT4 and stearoyl-CoA desaturase 2 genes (Kaestner et al., 1990; Christy et al., 1989). Thus, control of the expression of these genes by AA may be part of a generalized regulation of adipose-specific gene expression.

These in vitro results are supported by dietary experiments demonstrating that high safflower oil content diets (rich in ω-6 fatty acids) resulted in decreased cellular content and distribution of GLUT4 in rat adipocytes (Ezaki et al., 1992). Insulin-stimulated glucose transport activity was decreased to 51% of controls, and GLUT4 protein content of both plasma and microsomal membranes decreased by 35%. Fish oil feeding (increased ω-3 fatty acids) transiently improved the safflower oil-mediated decrease of insulin-stimulated glucose transport activity by increasing the amount of both GLUT1 and GLUT4 proteins. These data suggest that the regulatory properties of fatty acids may be determined by specific structure-function relationships.

Previous studies from our laboratory (Tebbey et al., 1994) examined the mechanisms by which AA can modulate glucose homeostasis in fully differentiated 3T3-L1 adipocytes. Chronic exposure of the adipocytes to 50 μM AA was demonstrated to alter both basal and insulin-stimulated glucose uptake and render the cells insulin resistant. The AA treatment specifically reduced the GLUT4 content of both the plasma and intracellular membranes, while GLUT1 content increased slightly. Consistent with the down-regulation of the protein, GLUT4 mRNA levels decreased to 10% of the initial content. Mechanistically the decrease was determined to be the result of both transcriptional down-regulation (50% of control) and a destabilization of the message (GLUT4 mRNA 1/2, decreased by 43%). These data suggested that AA, in a manner similar to insulin (Flores-Riveros et al., 1993a) and TNF (Stephens and Pekala, 1991, 1992), down-regulates expression of the insulin-responsive glucose transporter in adipocytes.

The oxidation of AA to very potent biological molecules is mediated by three different enzyme systems to include the cyclooxygenase, lipoxgenase, and cytochrome P-450 epoxyoxygenase. Thus, it became important to determine if oxidation of AA was necessary for its effect on GLUT4 or whether the observed effects were mediated by alternative pathways. In the current report we demonstrate that mechanistically fatty acids may act via two independent mechanisms, only one of which requires an oxidized metabolite of AA.

**EXPERIMENTAL PROCEDURES**

Materials—Dulbecco's modified Eagle's medium was purchased from Life Technologies, Inc. Fetal bovine serum was purchased from HyClone (Logan, UT) and used at a 1:10 dilution in Dulbecco's modified Eagle's medium. Based on specifications provided by HyClone, the mean AA content of culture medium containing 10% fetal bovine serum would be ~3 μM. Radiolabeled compounds were obtained from DuPont NEN. Hybond-N blotting membrane was purchased from Amersham Corp. Deoxyribonucleotides were obtained from Pharmacia Biotech Inc. KLewon fragment and TR1Zd Reagent was obtained from Life Technologies, Inc. The polycystic antiserum against PPARγ2 was developed against a peptide containing amino acids 284–298 as described by the clone, it was obtained from J. Anselmi of Affinity Bioreagents Inc. Tumor necrosis factor-α was the generous gift of Biogen (Cambridge, MA). The specific activity was 9.6 × 10⁶ units/mg, protein based on a cGMP assay using L929 cells. Prostaglandin E₂-Monoconal and cAMP Enzyme Immunoassay Kits were purchased from Cayman Chemical. Warialda acids and prostaglandins were purchased from Cayman Chemical. All other chemicals, unless otherwise stated, were of molecular biology grade and purchased from Sigma.

3T3-L1 Cell Culture—The murine 3T3-L1 cells used in this study were originally obtained from Dr. Howard Greene, Harvard University, Boston, MA. Cells were cultured, maintained, and differentiated as described previously (Tebbey et al., 1994). The cells were maintained for 10 days post-differentiation and then treated with the indicated agents for various times prior to RNA isolation.

Fatty Acids—All fatty acids used were absorbed onto diatomaceous earth and subsequently complexed to essentially fatty acid-free bovine serum albumin (BSA) to yield a FA:BSA ratio of ~1:4.1 (Tebbey and Buttke, 1992; Buttke et al., 1989). FA:BSA was added to cells from a 25 mM stock solution to yield a final concentration of 100 μM.

RNA Isolation and Northern Blot Analyses—Total RNA was isolated by extraction with guanidine isothiocyanate and centrifugation through a cesium chloride (Chirgwin et al., 1979) by the TRIzol Method (Life Technologies, Inc.). Northern analyses were performed as described previously (Stephens and Pekala, 1991).

DNA Probes—GLUT4, a 2.8-kilobase pair EcoRI fragment encoding the 3T3-L1 homolog of the adipose/muscle (insulin-responsive) glucose transporter (Kaestner et al., 1989); β-actin, a 1.9-kilobase pair HindIII fragment obtained from Dr. D. W. Cleveland (Cleveland et al., 1990).

Enzyme Immunoassay for PGE₂—Cells were treated with 100 μM AA/BSA for various times. At the indicated times 100 μl aliquots of medium were taken and analyzed for PGE₂ content using an enzyme immunoassay kit for PGE₂ (Cayman Chemical) based on the ELISA method.

Enzyme Immunoassay for cAMP—Cells were treated with 100 μM AA/BSA for various times and at the indicated times the cells were washed in phosphate-buffered saline and then scraped in 1 ml of 10% trichloroacetic acid. Cell debris was pelleted and cAMP was then extracted from the supernatant with an equal volume of 3:1 mixture of Freon-tri-n-ocylamine. The upper aqueous phase was collected (~500 μl) and then assayed for cAMP content using an enzyme immunoassay kit for cAMP (Cayman Chemical) based on the ELISA method.

Sphingomyelin Assay—Cells were labeled with [3H] choline chloride (specific activity of 1 M Ci/ml) at 0.5 μCi/ml for 6 days after initiation of differentiation, and the cells were grown in normal media for the remaining 3 days. On day 9 the cells were treated with either 100 μM AA or 5 μM TNF for the indicated times, the lipids extracted, and labeled sphingomyelin quantified by the bacterial sphingomyelinase method as described by J. Ajayev et al. (1994).

Preparation of Nuclear Extract—After the indicated treatments for various times, the cells were pelleted in phosphate-buffered saline for 10 min at 3000 rpm. The pellet was then resuspended in 3 ml of M3 lysate buffer (250 mM sucrose, 25 mM Tris, pH 7.8, 11 mM MgCl₂, 0.2% Triton X-100, 10 mM phenylmethylsulfonyl fluoride, 10 μM EDTA, 10 mM leupeptin, and 1 mg/ml aprotonin) and centrifuged at 10,000 rpm for 15 min. The nuclei were extracted using 250 μl of cold M4 extraction buffer (20 mM HEPES, pH 7.8, 0.4 mM KCl, 2 mM dithiothreitol, 20% glycerol, 0.5 mM phenylmethylsulfonyl fluoride, 10 μM EDTA, 10 mg/ml leupeptin, and 1 mg/ml aprotonin) for 30 min and then the nuclear debris and DNA pellet were assayed for protein concentrations by the method of Bradford (1976).

Electrophoretic Mobility Shift Assay (EMSA)—EMSAs were performed using the direct repeat half-site PPRE (5′-AATTCGGAGAACGTACCCTTTGCTTGCTGAGCAG-3′). Briefly, ~100 ng (60,000 cpm) of a 32P-labeled double-stranded DNA oligomer was incubated with 5 μg of nuclear extract for 15 min at room temperature in 10 μl of reaction buffer (25 mM Tris, pH 7.8, 0.5 mM EDTA, 88 mM KCl, 1 mM dithiothreitol, 100 μg of poly(dI-dC)-poly(dI-dC), 0.05% Triton X-100, and 12.5 μg salmon sperm DNA). Reaction mixtures were subjected to electrophoresis on a 5% nondenaturing polyacrylamide gel at 4°C, 40 amps (200 V), for ~2 h. The gels were dried and exposed to x-ray film for 12 h at ~80°C.

Statistical Analyses—The results of these experiments were analyzed using the Sigma-Stat package (Jandel Scientific Software, San Rafael, CA). Comparisons of means among groups were tested for significant differences by a Newman-Keuls procedure following one way-analysis of variance. The level of statistical significance chosen for these experiments was p < 0.05.

**RESULTS**

Inhibition of Cyclooxygenase Abolishes Arachidonic Acid-induced GLUT4 mRNA Suppression—AA is rapidly converted to a number of eicosanoids which express potent physiological properties. In order to identify involvement of either the lipo- or cyclooxygenase in the conversion of AA to a metabolite active in the suppression of GLUT4 gene expression, inhibitors of the two enzyme systems were used. Pretreatment of the 3T3-L1 adipocytes with nordihydroguaiaretic acid (NDGA), a selective inhibitor of the lipoxygenases (IC₅₀ = 0.2, 30, and 30 μM for 5-, 12-, and 15-LO, respectively, as opposed to 100 μM for the cyclooxygenase; Tobias and Hamilton, 1978) for 1 h at 50 μM did not significantly alter the AA-induced suppression of.
GLUT4 mRNA content (Fig. 1). In contrast, pretreatment with 50 μM indomethacin, which inhibits the cyclooxygenase (IC₅₀ = 0.1 μM; Tobias and Hamilton, 1978), for 1 h completely abolished the AA-induced decrease of GLUT4 mRNA content, consistent with conversion of AA to a prostanoid which in turn mediated suppression. Similar results were observed using the cyclooxygenase inhibitor ibuprofen (data not shown). While these results were rather compelling, a third oxidative pathway was considered. The cytochrome P-450 epoxygenase pathway has only been considered a major pathway in ocular and pituitary tissues (Fitzpatrick et al., 1981; Richelsen, 1987, 1992). Given the inhibition of indomethacin and ibuprofen on AA-induced GLUT4 mRNA suppression, we examined whether PGE₂ could elicit the same effect as AA. The results shown in Fig. 2, indicate that exposure of the cells for 12 h to 10 μM PGE₂ resulted in a marked decrease (−70%) in GLUT4 mRNA content. The effect was significantly (p < 0.05) more potent than either AA (−55%) or tumor necrosis factor-α (TNF) (−60%) decrease. Treatment of the cells with the nonmetabolizable analog of prostacyclin, carbaprostacyclin, for 12 h at 5, 10, or 25 μM had little or no effect on GLUT4 mRNA levels (data not shown), suggesting the specificity of PGE₂.

To further establish the role of PGE₂ in the regulation of GLUT4, as well as obtain support for the NDGA inhibitor data, we treated the cells with the hydroperoxy products of the lipoxygenase pathway, the 5(S)−, 12(S)−, and 15(S)-HPETEs for 12 h at 5 and 10 μM (Fig. 3). The data clearly demonstrated that, in contrast to PGE₂, these lipoxygenase metabolites had no effect on GLUT4 mRNA expression. To determine if AA was being converted to PGE₂ by the adipocytes, we measured PGE₂ levels present in the media at various times after the cells were treated with AA, using an ELISA. As shown in Fig. 4, within 1 h of AA addition to the cells, PGE₂ content increased 10-fold above basal levels. A maximum 50-fold increase above basal was observed 7 h after AA addition (Fig. 4). The duration of the PGE₂ peak was relatively short, lasting only 1 h and then returning slowly to basal levels over the next 15 h. These data suggest that AA is being converted via the cyclooxygenase pathway to PGE₂, the apparent active metabolite mediating GLUT4 suppression.

Cyclic AMP Increases in the 3T3-L1 Adipocytes with AA Treatment—PGE₂ functions to increase intracellular cAMP levels in various cells by stimulation of adenylate cyclase in an autocrine fashion (Davies and MacIntyre, 1992). In adipocytes, PGE₂ has been demonstrated to exhibit a concentration dependence with respect to its inhibitory or stimulatory effects on adenylate cyclase (Kather and Simon, 1977, 1979; Kather, 1981; Richelsen, 1987, 1992). Given the inhibition of indomethacin and ibuprofen on AA-induced GLUT4 mRNA suppression, we examined whether PGE₂ could elicit the same effect as AA. The results shown in Fig. 2, indicate that exposure of the cells for 12 h to 10 μM PGE₂ resulted in a marked decrease (−70%) in GLUT4 mRNA content. The effect was significantly (p < 0.05) more potent than either AA (−55%) or tumor necrosis factor-α (TNF) (−60%) decrease. Treatment of the cells with the nonmetabolizable analog of prostacyclin, carbaprostacyclin, for 12 h at 5, 10, or 25 μM had little or no effect on GLUT4 mRNA levels (data not shown), suggesting the specificity of PGE₂.

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To obtain a maximal response, based on preliminary studies, the cells were exposed to the fatty acid-albumin complex at a final concentration of 100 μM AA for 24 h. The results, displayed in Fig. 6, indicate that all fatty acids examined, stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3), γ-linolenic acid (γ18:3), mead fatty acid (20:3), AA (20:4), eicosapentaenoic acid (20:5), and the acetylenic analog of AA, ETYA, were able to suppress GLUT4 mRNA levels by at least 50% with AA providing the maximum suppression of 80%. While 18:2 and γ18:3 can be converted to AA and potentially lead to increased cAMP, none of the other fatty acids tested exhibit this potential. To confirm this, we examined the potentiation of 18:0, 18:1, and ETYA to increase cAMP, our data indicated that cAMP did not increase over the 5-h time frame of the experiment (mean ± S.D. of the [cAMP] over the 5-h time course, control: 178 ± 32 pmol/ml; 18:0-, 18:1-, and ETYA-treated: 149 ± 28, 139 ± 19, and 159 ± 21 pmol/ml, respectively).

Interestingly ETYA, which inhibits AA uptake, AA-specific and nonspecific acyl-CoA synthetases, the cyclooxygenase, and all lipooxygenases, PL}_{A2} as well as the cytochrome P-450 epoxygenase, and thus cannot be converted to PGE_{2}, was as effective as AA in suppressing GLUT4 mRNA accumulation. In addition, the suppression of GLUT4 mRNA accumulation could not be blocked by addition of indomethacin to the cultures. These studies are consistent with the premise that fatty acids may regulate GLUT4 mRNA content without the requirement for further metabolism and are consistent with the presence of a second signal transduction pathway.

AA Does Not Induce Sphingomyelin Turnover—Jayadev et al. (1994) have identified AA as one of several fatty acids that can activate the sphingomyelinase in HL-60 leukemia cells. This results in generation of free ceramide and initiation of a signal transduction cascade that potentially could control GLUT4 gene expression. To determine if this regulatory pathway was viable in the adipocytes, we examined the ability of AA to activate the sphingomyelinase and initiate sphingomyelin turnover. As shown in Fig. 7, after addition of AA to the cells, no significant hydrolysis of sphingomyelin was detected. TNF activation of the sphingomyelinase at a single 40-min time
point resulted in a 50% decrease in sphingomyelin and was used as a positive control. The data suggest that in the 3T3-L1 adipocytes, AA is not mediating activation of the sphingomyelinase and generation of free ceramide.

Involvement of Peroxisome Proliferator-activated Receptors (PPARs)—The newest members of the nuclear hormone receptor superfamily are the PPARs, which are known to be activated, in addition to the classic peroxisome activators such as clofibrate, by long chain saturated (Gottlicher et al., 1992; Gulick et al., 1994) and polyunsaturated fatty acids (Tontonoz et al., 1994a; Keller et al., 1993; Gottlicher et al., 1992). Thus, a predicted activity profile for an effect mediated by fatty acid activation of a PPAR would be similar to that displayed in Fig. 6 and discussed in the previous section. To establish a role for PPARs in the regulation of GLUT4 mRNA expression, fully differentiated 3T3-L1 cells were exposed to 0.5 and 1.0 mM clofibr acid for 12 h and GLUT4 mRNA levels were analyzed (Fig. 8). In a manner similar to AA and ETYA, 0.5 and 1.0 mM clofibr acid decreased GLUT4 mRNA content ~35 and 75%, respectively (the average area by quantification of phosphorimages was experimental variation; for control, 4450 ± 273; 0.5 mM clofibr, 2850 ± 140; 1.0 mM clofibr, 1187 ± 112). These data suggest that PPAR activation may result in GLUT4 mRNA suppression. PPARγ2, the predominant isoform expressed in adipose tissue (Tontonoz et al., 1994a; 1994b) has been shown to regulate the expression of adipocyte-specific genes and is activated by a diverse group of compounds including ETYA and fatty acids. In order to determine the potential for PPARγ2 involvement in the fatty acid-induced GLUT4 mRNA suppression, we performed two experiments using a DNA gel mobility shift assay (Fig. 9). Oligonucleotides containing the classic PPRE (TGAACCTTTGTGCT) from the promoter of the fatty acyl-CoA oxidase gene were end-labeled with [α-32P]dATP and then incubated with 10 μg of nuclear extracts. In the first experiment (Fig. 9A), nuclear extracts were prepared from the 3T3-L1 adipocytes and then treated with 50 μM AA, ETYA, and 10 μM retinoic acid. Separation of the complexes on a 5% nondenaturing polyacrylamide gel demonstrated that addition of AA or ETYA to the nuclear extracts results in a loss of binding of the PPAR to its responsive element. In the second experiment, nuclear extracts were prepared from cells that had been treated with 100 μM AA for 2 and 6 h or 100 μM ETYA for 12 h. Binding reactions were performed as described above. The results (Fig. 9B) demonstrate that treatment of the intact cells with these fatty acids markedly diminished protein binding to the PPRE. A partial supershift, performed with an antibody to PPARγ2, demonstrated that the protein-DNA complex was a heterodimer with one partner being PPARγ2 (data not shown). The data from the two experiments suggest that both AA and its nonmetabolizable analog are capable of disrupting the occupancy of a PPAR and that neither protein synthesis nor oxidation to PGE2 is necessary for the alteration of binding.

DISCUSSION

To address the mechanism by which AA can suppress GLUT4 gene expression, we have investigated the metabolism and the signal transduction properties of AA in the 3T3-L1 adipocytes. Our studies have demonstrated that the major metabolic fate of exogenously added AA is the oxidative metabolism through the cyclooxygenase pathway to a prostanoid.
These conclusions are based on the data demonstrating that the effect of AA on GLUT4 can be blocked by indomethacin, a cyclooxygenase inhibitor, while NDGA, a lipoxygenase inhibitor, cannot inhibit the effect of AA. Of the prostanoids derived from AA, isolated adipocytes have been demonstrated to produce only the prostaglandins, PGE$_2$ and PGI$_2$ (prostacyclin) in considerable amounts (Axelrod and Levine, 1981; Richelsen, 1987). To determine if either of these compounds contributed to the suppression of GLUT4, the fully differentiated 3T3-L1 adipocytes were exposed to PGE$_2$ and carbaprostacyclin, the chemically stable analog of prostacyclin. Prostaglandin E$_2$ treatment resulted in an 70% decrease in GLUT4 mRNA levels, while carbaprostacyclin had no effect on GLUT4 mRNA content. Quantification of the conversion of AA to PGE$_2$ demonstrated a 50-fold increase in PGE$_2$ released into the media within 3 h of AA addition (Fig. 3). These data, demonstrating the time course and magnitude of PGE$_2$ formation, are consistent with PGE$_2$ synthesis as being the major metabolic fate of exogenously added AA. Moreover, they suggest that PGE$_2$ may be an intermediate in regulation of GLUT4 gene expression. In a number of systems, the oxidative metabolism of AA appears to be a requirement to derive the metabolite capable of regulating specific gene expression. In Swiss 3T3 fibroblasts, AA was shown to induce c-fos and Egr-1 mRNA through formation of PGE$_2$ and subsequent activation of protein kinase C (Danesch et al., 1994). Lipoxygenase metabolites have also been shown to stimulate the accumulation of c-fos and c-jun mRNA in human monocytes while they increase c-fos accumulation in quiescent TA1 cells (Haliday et al., 1991). While underlying mechanisms have not as of yet been identified, these reports demonstrate a role for AA and its metabolites in the regulation of specific genes. Prostanoids are local hormones that once released from the cell bind to cell surface receptors to act in an autocrine or paracrine fashion. Depending on the cell type, there are three distinct receptors for PGE$_2$. Ligand occupation of the appropriate receptor can lead to activation or inhibition of adenylate cyclase, each mediated through a specific G-protein, while occupation of the third class of receptors can stimulate Ca$^{2+}$ mobilization and subsequent activation of protein kinase C (Davies and Madinhyre, 1992). Interestingly, the 3T3-L1 adipocytes have recently been shown to lack the Ca$^{2+}$-activated protein kinase C isoforms (McGowan et al., 1996) and thus a mechanism dependent on PGE$_2$ activation of protein kinase C is ruled out. Our data are consistent with a cAMP-mediated mechanism with cAMP levels rising 50-fold above basal (10 $\mu$m) within 3 h of AA addition. The decrease in cAMP content, with levels returning to basal within 30 min while PGE$_2$ levels remained markedly elevated, suggest a rapid desensitization of the PGE$_2$ receptor to adenylate cyclase coupling mechanism.

We (Stephens and Pekala, 1992) and others (Ezaki et al., 1993; Flores-Riveros, et al., 1993b) have demonstrated the ability of cAMP to suppress transcription of the GLUT4 gene, leading to a decrease in the content of GLUT4 mRNA. Stephens and Pekala (1992) demonstrated that the effect of 8-bromo-cAMP on GLUT4 transcription was rapid, with a 40% decrease in rate detected within 1 h and a 52% decrease within 4 h. With respect to both magnitude and temporal considerations, these results were very similar to those we have reported for AA, where a 50% decrease in GLUT4 gene transcription occurred in response to AA treatment (Tebbey et al., 1994). Studies by Flores-Riveros et al. (1993b) indicated that the regulatory element mediating transcriptional repression by cAMP resides in the proximal promoter of the GLUT4 gene between positions −469 and −78. Thus, based on these observations it is likely that a cAMP-dependent protein kinase is involved in the transcriptional repression of the GLUT4 gene where an interaction between the cis-regulatory element and the activity of a trans-acting factor is regulated by cAMP-mediated phosphorylation.

Examination of a diverse family of fatty acids for the specificity of the AA effect demonstrated that suppression of GLUT4 was not limited to AA (Fig. 6). Perhaps the strongest evidence for an alternative pathway is that ETYA, a nonmetabolizable AA analog reported to be a potent inhibitor of AA transport and metabolism (Tobias and Hamilton, 1978), is nearly as potent as AA in its ability to suppress GLUT4 mRNA accumulation. These data would suggest that the fatty acids can function as a regulator of GLUT4 mRNA expression by a pathway that does not involve oxidative metabolism.

Our previous studies with AA demonstrated that in addition to a suppression of transcription, exposure of the adipocytes to AA resulted in a destabilization of the GLUT4 mRNA (t$_{1/2}$ decreased from 9.3 to 4.5 h). However, in the studies described above (Stephens and Pekala, 1992; Ezaki et al., 1993) no effect was observed on the stability of the GLUT4 mRNA when the cells were exposed to 8-bromo-cAMP, thereby localizing the effect on transcription to CAMP. These data taken collectively suggest that AA must initiate a second signal transduction cascade that is responsible for the alteration of GLUT4 mRNA stability. In an attempt to further define this issue, we examined whether ETYA or 18:1 could alter GLUT4 mRNA stability. After exposure of the cells to ETYA for 24 h, the half-life of the GLUT4 mRNA was determined to be 8.1 ± 1.1 h (n = 3). This did not test significantly different than control. However, incubation of the cells in the presence of 18:1 resulted in a stability
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