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Synergistic Effect of Prostaglandin F2α and Cyclic AMP on Glucose Transport in 3T3-L1 Adipocytes

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Abstract The combined effect of prostaglandin F2α (PGF2α) and cAMP on glucose transport in 3T3-L1 adipocytes was examined. In cells pretreated with PGF2α and 8-bromo cAMP for 8 h, a synergy between these two agents on glucose uptake was found. Insulin-stimulated glucose transport, on the other hand, was only slightly affected. The synergistic effect of these two agents was suppressed in the presence of cycloheximide and actinomycin D. In concord, immunoblot and Northern blot analyses revealed that GLUT1 protein and mRNA levels were both increased in cells pretreated with both PGF2α and 8-bromo cAMP, greater than the additive effect of each agent alone. The synergistic action of PGF2α with 8-bromo cAMP to enhance glucose transport was inhibited by GF109203X, a selective protein kinase C (PKC) inhibitor. In addition, in cells depleted of diacylglycerol-sensitive PKC by prolonged treatment with 4β-phorbol 12β-myristate 13α-acetate, a PKC activator, the synergistic effects of PGF2α and 8-bromo cAMP on glucose transport and GLUT1 mRNA accumulation were both abolished. Taken together, these results indicate that PGF2α may act with cAMP in a synergistic way to increase glucose transport, probably through enhanced GLUT1 expression by a PKC-dependent mechanism. J. Cell. Biochem. 94: 627–634, 2005.

Key words: prostaglandin F2α; 3T3-L1 adipocytes; glucose transport; protein kinase C; GLUT1; cyclic AMP

Prostaglandin F2α (PGF2α) is a cyclooxygenase metabolite of arachidonic acid (AA) [Sales and Jabbour, 2003]. Besides its well-known role in reproductive physiology [Krzymowski and Stefanczyk-Krzymowska, 2002; Pate, 2003], PGF2α is also found to act as an autocrine/paracrine modulators of osteoblasts [Nijweide et al., 1986], to modulate the growth and the differentiation of bovine corneal epithelial cells [Conconi et al., 2001], and to inhibit adipocyte differentiation [Miller et al., 1996; Kamon et al., 2001]. In addition, PGF2α may modulate glucose metabolism; it was found to induce GLUT1 expression and glucose uptake in neonatal cardiomyocytes [Morissette et al., 2003] and in differentiated 3T3-L1 adipocytes [Chiou and Fong, 2004].

We have demonstrated earlier that AA as well as endothelin-1 may act with cAMP in a synergistic way to increase glucose uptake in 3T3-L1 adipocytes by a protein kinase C (PKC)-dependent mechanism [Fong et al., 1999, 2004]. Cyclooxygenase metabolites of AA, however, do not seem to be involved in the synergistic effect of AA and cAMP on glucose transport [Fong et al., 1999]. Since PGF2α may induce glucose transport in 3T3-L1 adipocytes by a PKC-dependent mechanism [Chiou and Fong, 2004], it is tempting to test if there is also a synergism between PGF2α and cAMP on glucose transport in 3T3-L1 adipocytes. The results of this study demonstrated that it is indeed the case. In addition, the enhanced glucose transport in cells exposed to both PGF2α and cAMP is mainly attributed to a PKC-dependent increase in GLUT1 expression.

MATERIALS AND METHODS

Materials

Dexamethasone, 3-isobutyl-1-methylxanthine (IBMX), 2-deoxy-D-glucose (2-deoxyglucose), cycloheximide, phenylmethylsulfonyl fluoride (PMSF), cytochalasin B, staurosporine, actinomycin D, 4β-phorbol 12β-myristate 13α-acetate

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(PMA), GF109203X, PGF2α, and insulin were obtained from Sigma Chemicals (St. Louis, MO). [α-^{32}P]dCTP (3000 Ci/mmol) was from Amersham (Arlington Height, IL). [^{3}H]2-deoxyglucose (8 Ci/mmol) was obtained from New England Nuclear (Boston, MA).

Cell Culture

3T3-L1 cells, obtained from American type culture collection, were grown in 10-cm dishes (Corning, Acton, MA) or 12-well plates (Costar, Acton, MA) containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS; Gibco BRL, Carlsbad, CA), glutamine, penicillin, and streptomycin as described [Carnicero, 1984]. The cultures were kept at 37°C in a humidified atmosphere of 10% CO_2, 90% air, and the medium was changed every 2 days. 3T3-L1 preadipocytes were differentiated into adipocytes according to the procedure as described [Carnicero, 1984; Rubin et al., 1978] with modification [Fong, 1990]. Briefly, 2 days after confluence (day 0), the medium was removed and fresh medium containing 0.5 mM IBMX and 0.25 mM dexamethasone was added. After another 3 days, the medium was replaced with fresh culture medium and the cultures were then maintained as described above. By day 8, more than 90% of the cells have differentiated into rounded cells with lipid droplets.

Preparation of Membrane Fractions

Total cellular membranes were prepared as described earlier [Fong et al., 1996]. Briefly, cells were treated with tested reagents for indicated times, washed twice with phosphate-buffered saline (PBS), and homogenized in Buffer A (250 mM sucrose, 20 mM HEPES, 1 mM EDTA, 1 mM PMSF, and 1 μg/ml each of aprotinin, pepstatin, and leupeptin, pH 7.4) on ice with a motor-driven Teflon-coated pestle. Following centrifugation at 600*g* for 10 min to remove cell debris and fat cake, subsequent centrifugation at 100,000*g* for 1 h resulted in membrane fractions in the pellet. The membranes were resuspended to about 5 mg of protein/ml in buffer B (20 mM HEPES, 1 mM EDTA, and 1 mM PMSF, pH 7.4). All centrifugation was performed at 4°C.

Immunoblot Analysis

For each experiment, membrane samples were subjected to SDS–polyacrylamide (10%) gel electrophoresis (SDS–PAGE) under reducing conditions as described by Laemmli [1970], and transferred to a nitrocellulose membrane. The GLUT1 and GLUT4 transporter proteins were immunodetected by using polyclonal rabbit anti-GLUT1 (1:1,000; East Acres Biologicals, Southbridge, MA) and monoclonal mouse anti-GLUT4 (IF8, 1.3 μg/ml; Genzyme, Cambridge, MA) antibodies, respectively, and the blots were developed by the enhanced chemiluminescence method (ECL, Amersham, Buckinghamshire, UK) employing horseradish peroxidase-conjugated donkey anti-rabbit IgG (1:5,000) for GLUT1 and sheep anti-mouse IgG (1:9,000) for GLUT4, respectively. Quantification of relative band intensity was performed by laser scanning densitometry.

Preparation of Total RNA and Northern Blot Analysis

Total cellular RNA was isolated by a TRI REAGENT extraction kit (Molecular Research Center, Inc., Cincinnati, OH). For Northern blot analysis, 40 μg of RNA isolated from 3T3-L1 adipocytes following various treatments was denatured with formamide/formaldehyde and resolved by 1% formaldehyde-agarose gel electrophoresis. After electrophoresis, RNA was transferred to a Hybond-N-nylon membrane (Amersham) and cross-linked to the membrane with UV light. A cDNA probe for GLUT1 was radiolabeled by Rediprime DNA Labeling System (Amersham Life Science) and hybridization was performed as described by the manual. The radioactivity was detected by exposing the nylon paper to a Kodak X-Omat AR film for a suitable length of time and the extent of hybridization quantified by laser scanning densitometry.

Preparation of GLUT1 cDNA Probe by Polymerase Chain Reaction

ThermoScript RT-PCR System (Gibco BRL) was employed to prepare mouse GLUT1 cDNA probe. Briefly, in the first step, cDNA synthesis was performed at 65°C using 5 μg of total RNA of 3T3-L1 adipocytes as the template primed with random hexamers. In the second step, an aliquot of the cDNA was subjected to PCR amplification using Platinum™ *Tag* DNA polymerase in the presence of a pair of primers, forward primer (5′-CGTGCTCTTCTTCATCTTCACCTAC-3′) and reverse primer (5′-GTCTTCAGCAGTTAAGTTCTCAGCC-3′), covering the

region from nucleotide 1512 to 2377 of the mouse GLUT1 cDNA. PCR was carried out under the following conditions: 96°C(5 min) → [60°C(2 min) → 72°C(4 min) → 96°C(2 min)] × 34 cycles → 72°C (60 min). The product, 866 bp in length, was excised from a 1% agarose gel after electrophoresis and recovered by electroelution. The cDNA probe was sequenced and confirmed to be mouse GLUT1 fragment.

Measurement of Glucose Transport Activity

Glucose transport activity was analyzed by measuring uptake of 2-deoxyglucose into the cells as described previously [Chu et al., 2002]. Briefly, after the cells were treated with the tested reagents for appropriate times, [^{3}H]2-deoxyglucose(final concentration 0.2 mM, 7.5 μCi/μmol) was added and incubation was continued for another 5 min. The reaction was terminated by the addition of cold (0°C) PBS also containing 20 mM D-glucose. Cells were washed three times with the same buffer, lyzed with 2% SDS, and counted for radioactivity. Carrier-specific uptake was obtained by correction for nonspecific diffusion of [^{3}H]2-deoxyglucose into the cells as estimated in the presence of 10 μM cytochalasin B, which accounted for approximately 6%–8% of the activity. Statistical differences were determined by Student's *t*-test.

RESULTS AND DISCUSSION

Synergistic Effect of PGF2α and cAMP on Glucose Uptake

The combined effect of PGF2α and 8-bromo cAMP on 2-deoxyglucose uptake was measured in the presence of varying concentrations of 8-bromo cAMP and 1 μM PGF2α for 8 h. As shown in Figure 1, a synergism between PGF2α and 8-bromo cAMP was observed. Although 8-bromo cAMP at 0.25 mM had only a small enhancing effect on 2-deoxyglucose uptake (↑60% over control, $P < 0.05$), in the presence of 1 μM PGF2α, which alone increased 2-deoxyglucose uptake to approximately 255% of control ($P < 0.01$), the combined effect of 8-bromo cAMP and PGF2α increased 2-deoxyglucose uptake to approximately 610% of control ($P < 0.01$). The highest synergistic effect of 8-bromo cAMP with PGF2α was observed at 0.5 mM. At 0.5 mM 8-bromo cAMP and 1 μM PGF2α, 2-deoxyglucose uptake was increased to approximately 865% of control ($P < 0.01$), much higher than the theoretical additive effect of either agent alone (295% of control for 8-bromo cAMP, $P < 0.01$ and 255% of control for PGF2α, $P < 0.01$, respectively). Since the greatest synergism was observed between 0.5 mM 8-bromo cAMP and 1 μM PGF2α, these concentrations were used throughout this study to investigate the mechanism responsible for the synergistic effect of PGF2α and cAMP on glucose transport. The insulin-stimulated glucose transport, on the other hand, was not significantly influenced by pre-treating cells with a combination of PGF2α and 8-bromo cAMP for 8 h (Table I).

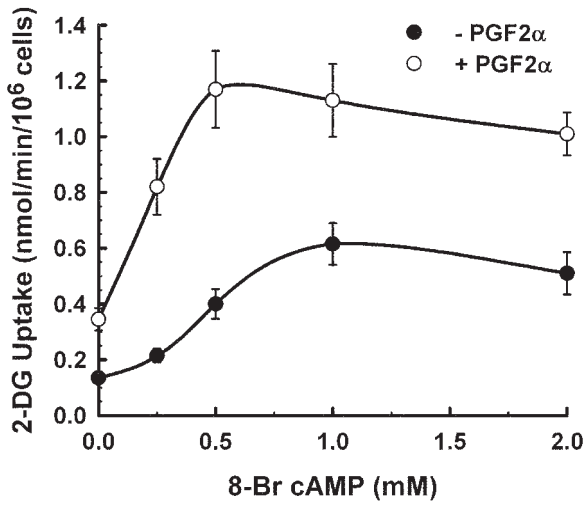

Fig. 1. Combined effect of prostaglandin F2α (PGF2α) and 8-bromo cAMP on glucose transport. 3T3-L1 adipocytes were treated with various doses of 8-bromo cAMP alone or in combination with 1 μM PGF2α for 8 h, washed and measured for 2-deoxyglucose (2-DG) uptake as described in "Materials and Methods." Values are mean ± SE from three separate experiments with triplicate determinations.

Effect of Inhibitors of Protein Synthesis and Gene Transcription on the Synergistic Effect of PGF2α and 8-Bromo cAMP on Glucose Transport

To examine if de novo protein synthesis was involved in the synergism between PGF2α and cAMP, the inhibitory effect of cycloheximide was determined. As shown in Table II, when protein synthesis was arrested in the presence of cycloheximide, the stimulatory effect of PGF2α alone and the synergistic effect of PGF2α and cAMP on 2-deoxyglucose uptake were completely inhibited, implying the requirement for a continued protein synthesis. The enhancing effect of 8-bromo cAMP on glucose uptake, on the other hand, was only inhibited by 39% ($P < 0.02$), suggesting that PGF2α and cAMP

TABLE I. Combined Effect of PGF2α and 8-Bromo cAMP on Basal and Insulin-Stimulated Glucose Uptake

	2-DG uptake (nmol/min/10^6 cells)		
	Basal	Insulin	Δ(Insulin-basal)
Control	0.170 ± 0.020	1.350 ± 0.110**	1.180 ± 0.083
PGF2α	0.409 ± 0.030*	1.589 ± 0.070**	1.180 ± 0.050
8-bromo cAMP	0.361 ± 0.040*	1.430 ± 0.090**	1.069 ± 0.066
PGF2α + 8-bromo cAMP	1.380 ± 0.102*	2.600 ± 0.150*,**	1.220 ± 0.122

3T3-L1 adipocytes were pretreated with 1 μM PGF2α, 0.5 mM 8-bromo cAMP, or a combination of both for 8 h, washed and then measured for basal or insulin-stimulated 2-deoxyglucose (2-DG) uptake as described in "Materials and Methods." Values are mean ± SE (n = 3).
*$P < 0.05$ or less compared to its corresponding control.
**$P < 0.01$ or less compared to its corresponding basal.

seem to exert their influence on glucose transport through distinct mechanisms.

The possibility of involvement of gene transcription was tested by using a transcription inhibitor actinomycin D. As shown in Table II, in the presence of actinomycin D, basal 2-deoxyglucose uptake was increased by approximately twofold ($P < 0.01$) and the ability of 8-bromo cAMP to induce glucose uptake was minimally affected (↓11%, $P > 0.05$). Conversely, in cells pretreated with actinomycin D, the ability of PGF2α to further stimulate glucose transport was inhibited by 91% ($P < 0.01$) and the synergistic effect of PGF2α and 8-bromo cAMP on glucose transport was nearly abolished. Thus involvement of gene transcription may not be needed for cAMP to exert its stimulatory effect, but is essential for the synergistic effect of PGF2α and cAMP on glucose transport. In addition, inhibition of transcription by actinomycin D has been consistently found to increase glucose uptake in adipocytes [Chiou and Fong, 2004; Fong et al., 2004], but not in muscles [Kawanaka et al., 2001], implying that this is not a generous phenomenon. The mechanism, however, remains unknown.

Analysis of GLUT1 Protein Content and GLUT1 mRNA Accumulation in Cells Pretreated With PGF2α and 8-Bromo cAMP

Since the synergistic effect of PGF2α and 8-bromo cAMP on glucose transport seemed to involve both protein synthesis and gene transcription (see above), we further assessed if increased levels of GLUT1 protein and mRNA were responsible for the augmented glucose transport in cells exposed to both PGF2α and cAMP. As shown in Figure 2, after 8-h exposure to PGF2α, 8-bromo cAMP and both, the content of GLUT1 protein in total membrane fractions was increased to approximately 182 ± 30%, 175 ± 26%, and 385 ± 63% of control (mean ± SE SE from three separate experiments as determined by densitometry), respectively. The doublet protein bands shown in gels probably represent GLUT1 proteins with different extent of glycosylation [Haspel et al., 1985, 1988]. In agreement with the result of Table I, GLUT4

TABLE II. Effect of Cycloheximide (CHX) and Actinomycin D on Glucose Transport in Response to PGF2α, 8-Bromo cAMP, or a Combination of Both

	2-DG uptake (nmol/min/10^6 cells)		
	None	CHX	Actinomycin D
Control	0.161 ± 0.013	0.157 ± 0.008	0.332 ± 0.015**
PGF2α	0.365 ± 0.018*	0.154 ± 0.003**	0.351 ± 0.013
8-bromo cAMP	0.436 ± 0.028*	0.324 ± 0.026*,**	0.578 ± 0.035*,**
PGF2α + 8-bromo cAMP	1.305 ± 0.079*	0.312 ± 0.028*,**	0.610 ± 0.042*,**

After 3T3-L1 adipocytes were pretreated with none, 10 μg/ml CHX or 5 μg/ml actinomycin D for 30 min, vehicle (control), 1 μM PGF2α, 0.5 mM 8-bromo cAMP, or a combination of both were added, and the incubation was continued for another 8 h. Cells were then washed and measured for 2-deoxyglucose (2-DG) uptake as described in "Materials and Methods." Values are mean ± SE (n = 3).
*$P < 0.01$ or less compared to its corresponding control.
**$P < 0.05$ or less compared to its corresponding none (without CHX or actinomycin D treatment).

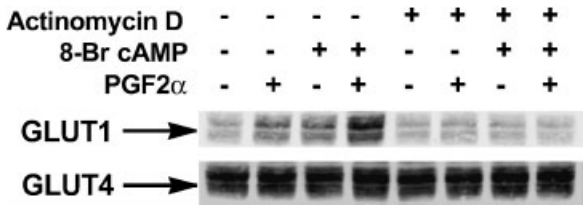

Fig. 2. Immunoblot analysis of GLUT1 and GLUT4 protein content in total membranes. After 3T3-L1 adipocytes were pretreated without or with 5 μg/ml actinomycin D for 30 min, vehicle, 1 μM PGF2α, 0.5 mM 8-bromo cAMP, or PGF2α + 8-bromo cAMP were added for another 8 h. Total cellular membranes were isolated and subjected to SDS–PAGE and immunoblotting using antibody against GLUT1 or GLUT4 as described in ''Materials and Methods.'' Two other separate experiments gave similar results.

protein content was not altered under the same experimental conditions. Simultaneous presence of actinomycin D, on the other hand, abolished the increase in GLUT1 content induced by PGF2α, 8-bromo cAMP, or a combination of both, implying that the increased amount of GLUT1 is mainly accounted for by increased Glut1 transcription. To further substantiate this notion, time-dependent effects of PGF2α, 8-bromo cAMP, and both on GLUT1 mRNA accumulation were examined. As shown in Figure 3, whereas PGF2α or 8-bromo cAMP alone gradually increased the level of GLUT1 mRNA in a time-dependent manner, a combination of both seemed to expedite the process and dramatically enhanced the GLUT1 mRNA levels. In cells exposed to PGF2α, 8-bromo cAMP, or both for 8 h, GLUT1 mRNA accumulations were increased to $330 \pm 40\%$, $375 \pm 65\%$, and $960 \pm 105\%$ of control (time = 0) (mean ± SE from three separate experiments using GLUT1/18S ratio for comparison), respectively. These results thus suggest that the greater increase in glucose transport activity in cells treated with both PGF2α and 8-bromo cAMP may result from larger amount of GLUT1 protein, which is derived from an augmented increase in GLUT1 mRNA level.

Involvement of PKC in the Synergistic Effect of PGF2α and cAMP on Glucose Transport

We have demonstrated earlier that PKC activator PMA may act with cAMP in a synergistic fashion to induce GLUT1 transcription as well as glucose transport in 3T3-L1 adipocytes [Fong et al., 2004], implying that at least one diacylglycerol-sensitive PKC isoform is involved in the interaction with cAMP pathway. Although PKC seems to mediate PGF2α's diversified effects in a variety of cells [Tokuda et al., 1999; Kanashiro et al., 2000; Chen et al., 2001] and PGF2α was able to induce glucose transport by a PKC-dependent pathway in 3T3-L1 adipocytes [Chiou and Fong, 2004], it is not necessary that PKC is absolutely involved in the synergistic effect of PGF2α and cAMP on glucose transport, if the PKC isoform responsible for the synergistic interaction with cAMP on glucose transport is not the one that can be activated by PGF2α. Only five PKC isoforms (PKCα, βII, γ, δ, and ε) were found responsive to PMA in 3T3-L1 adipocytes [Fong et al., 2004], and PGF2α was shown to activate PKCε but had no effect on PKCα or PKCγ [Chiou and Fong, 2004]. The ability of PGF2α to activate PKCδ or PKCβII was not determined. We therefore tested the possibility of PKC involvement in the synergistic interaction between PGF2α and cAMP by using PKC inhibitors and prolonged PMA-pretreatment to deplete diacylglycerol-sensitive PKC. As shown in Table III, both staurosporine, a non-specific PKC inhibitor [Way et al., 2000]

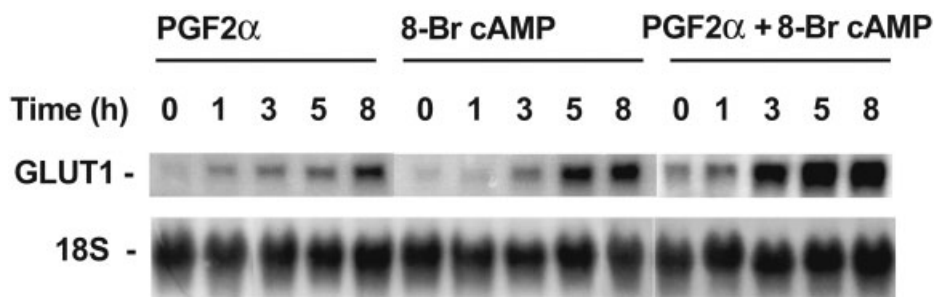

Fig. 3. Time-dependent effect of PGF2α, 8-bromo cAMP, and a combination of both on GLUT1 mRNA accumulation. After cells were treated with 1 μM PGF2α, 0.5 mM 8-bromo cAMP, or PGF2α + 8-bromo cAMP for various times, GLUT1 mRNA accumulation was estimated by Northern blot analysis as described in ''Materials and Methods.'' 18S RNA was used as the internal control for normalization. Two other separate experiments gave similar results.

TABLE III. Effects of Staurosporine and GF109203X on Glucose Transport in Response to PGF2α, 8-Bromo cAMP, or a Combination of Both

	2-DG uptake (nmol/min/10^6 cells)		
	None	Staurosporine	GF109203X
Control	0.125 ± 0.010	0.143 ± 0.009	0.102 ± 0.009
PGF2α	0.316 ± 0.020*	0.195 ± 0.023**	0.136 ± 0.010**
8-bromo cAMP	0.294 ± 0.019*	0.264 ± 0.032*	0.234 ± 0.021*
PGF2α + 8-bromo cAMP	1.055 ± 0.094*	0.310 ± 0.040*,**	0.376 ± 0.056*,**

After 3T3-L1 adipocytes were pretreated with none, 100 nM staurosporine or 1 μM GF109203X for 1 h, vehicle (control), 1 μM PGF2α, 0.5 mM 8-bromo cAMP, or a combination of both were added, and the incubation was continued for another 8 h. Cells were then washed and measured for 2-deoxyglucose (2-DG) uptake as described in "Materials and Methods." Values are mean ± SE (n = 3).
*$P < 0.05$ or less compared to its corresponding control.
**$P < 0.05$ or less compared to its corresponding none (without staurosporine or GF109203X treatment).

and GF109203X, a selective PKC inhibitor [Toullec et al., 1991], inhibited the synergistic effect of PGF2α and 8-bromo cAMP on 2-deoxyglucose uptake by approximately 100% ($P < 0.01$) and 81% ($P < 0.01$), respectively. In agreement with our earlier finding [Chiou and Fong, 2004], both inhibitors greatly decreased glucose transport in response to PGF2α (↓73% for staurosporine, $P < 0.02$ and ↓82% for GF109203X, $P < 0.01$, respectively), but had much less influence on 8-bromo cAMP-induced glucose uptake (↓28%, $P > 0.05$ and ↓22%, $P > 0.05$, respectively). These results thus suggest that the synergistic effect of PGF2α and cAMP on glucose transport involves a PKC-dependent mechanism. This notion is further substantiated by the observations that in cells depleted of diacylglycerol-sensitive PKC by prolonged pretreatment with PMA, the synergistic effects of PGF2α and 8-bromo cAMP on glucose transport (Table IV) and GLUT1 mRNA accumulations (Fig. 4) were both nearly abolished. The observation of an increased basal glucose transport (↑38%, $P < 0.05$) and augmented glucose uptake in response to 8-bromo cAMP (↑114%, $P < 0.02$) in PMA-pretreated cells (Table IV) is in agreement with our earlier finding that initial activation of PKC by PMA is sufficient to transmit the signal for enhancing glucose transport as well as for the synergism with cAMP [Fong et al., 1999].

In summary, we have demonstrated that PGF2α and cAMP may act in a synergistic way to enhance glucose uptake into 3T3-L1 adipocytes, mainly through enhanced GLUT1 expression, via a PKC-dependent mechanism. Although it is not certain which PKC isoform is involved, based on the findings that both PGF2α and endothelin-1 act similarly with cAMP in a synergistic way to induce glucose transport by a PKC-dependent mechanism and endothelin-1 can activate only PKCε and PKCδ among the five PMA-responsive PKC isoforms in 3T3-L1 adipocytes [Fong et al., 2004], it is likely that PKCε, the one that can be activated by both PGF2α and endothelin-1, is the isozyme involved. Nevertheless, we cannot exclude the possibility that PKCδ or both PKCε and PKCδ are actually involved.

It is interesting to note that a combination of PGF2α and cAMP may induce glucose transport to a level comparable to that induced by insulin (Table I), although distinct mechanisms are apparently involved. While the effect of insulin on glucose transport is fast and is mainly ascribed to the translocation of GLUT4 from intracellular pools to plasma membranes

TABLE IV. Effect of Prolonged 4β-Phorbol 12β-Myristate 13α-Acetate (PMA)-Pretreatment on Glucose Transport in Response to PGF2α, 8-Bromo cAMP, or a Combination of Both

	2-DG uptake (nmol/min/10^6 cells)	
	None	PMA-pretreated
Control	0.135 ± 0.011	0.186 ± 0.014**
PGF2α	0.312 ± 0.018*	0.239 ± 0.018**
8-bromo cAMP	0.297 ± 0.023*	0.533 ± 0.056*,**
PGF2α + 8-bromo cAMP	0.855 ± 0.072*	0.610 ± 0.051*,**

After 3T3-L1 adipocytes were pretreated with none or 1 μM PMA for 16 h, vehicle (control), 1 μM PGF2α, 0.5 mM 8-bromo cAMP, or a combination of both was added, and the incubation was continued for another 8 h. Cells were then washed and measured for 2-deoxyglucose (2-DG) uptake as described in "Materials and Methods." Values are mean ± SE (n = 3).
*$P < 0.01$ or less compared to its corresponding control.
**$P < 0.05$ compared to its corresponding none (without PMA-pretreatment).

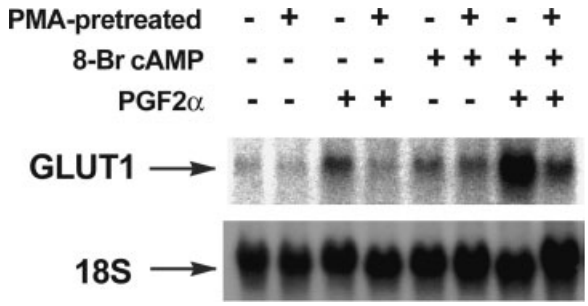

Fig. 4. Effect of prolonged 4β-phorbol 12β-myristate 13α-acetate (PMA)-pretreatment on GLUT1 mRNA accumulation in response to PGF2α, 8-bromo cAMP, and a combination of both. After 3T3-L1 adipocytes were pretreated without or with 1 μM PMA for 16 h, vehicle, 1 μM PGF2α, 0.5 mM 8-bromo cAMP, or PGF2α + 8-bromo cAMP were added for another 8 h. Total RNA was isolated and analyzed for the content of GLUT1 mRNA as described in ''Materials and Methods.'' 18S RNA was used as the internal control. These experiments were repeated twice with similar results.

[Birnbaum, 1992], the effect of a combination of PGF2α and cAMP is relatively slow and involves transcription and translation of GLUT1. Thus it appears that high glucose flux into adipocytes may be achieved by both GLUT4- and GLUT1-mediated transport. The circumstances for glucose transport in response to insulin or a combination of PGF2α and cAMP, however, are quite different. For example, insulin-stimulated glucose uptake into adipocytes is accompanied with suppressed lipolysis, whereas a combination of PGF2α and cAMP seems to enhance both glucose uptake and lipolysis. As a consequence, some of the liberated fatty acids as induced by cAMP may be redirected back to triglycerides via increased levels of glycerol-3-phosphate. The physiological significance of this phenomenon, nevertheless, needs to be further explored.

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