Further Evidence Implicating Diacylglycerol Generation and Protein Kinase C Activation in Agonist-Induced Increases in Glucose Uptake

Insulin-Like Effects of Phenylephrine in BC3H-1 Myocytes

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SUMMARY

We have previously suggested that insulin effects on 2-deoxyglucose (2-DOG) uptake in BC3H-1 myocytes are due to increases in de novo phospholipid synthesis, diacylglycerol generation, and protein kinase C activation. To test this hypothesis further, we examined the effects of phenylephrine, an agonist that increases diacylglycerol and protein kinase C activity through phospholipase C activation. As evidence for phospholipase activation in BC3H-1 myocytes, we found that phenylephrine increased *1)* acute $^{32}PO_4$ incorporation into phosphatidic acid and phosphatidylinositol, *2)* generation of [^{3}H]inositol phosphates from prelabeled [^{3}H]inositol phospholipids, *3)* cytosolic Ca^{2+}, and *4)* membrane-bound protein kinase C. Phenylephrine also provoked dose-related increases in [^{3}H]2-DOG uptake that were similar in magnitude and time course to those induced by insulin. As with insulin, phenylephrine effects on 2-DOG uptake were not apparent in myocytes that were maximally stimulated with 12-*O*-tetradecanoylphorbol-13-acetate, a diacylglycerol analogue that activates protein kinase C. These findings support our hypothesis that diacylglycerol generation and protein kinase C activation may be important in the stimulation of glucose uptake by agents such as phenylephrine and insulin that activate the phosphoinositide cycle. DIABETES 1986; 35:951–57.

We have previously reported that 12-*O*-tetradecanoylphorbol-13-acetate (TPA) has insulin-like effects on glucose uptake, amino acid uptake, and pyruvate dehydrogenase activation in BC3H-1 myocytes.[1] Because insulin increases diacylglycerol (DAG) content in these myocytes[2] and because protein kinase C is activated by both DAG[3] and TPA,[4] we made the initial postulation that insulin may enhance glucose uptake by a mechanism involving DAG generation and protein kinase C activation. We also proposed that the insulin effect on DAG was for the most part due to rapid increases in de novo phospholipid synthesis[2,5] rather than to activation of phospholipase C because we did not observe insulin-induced increases in inositol-phospholipid hydrolysis, inositol (mono-, di-, or tri-)phosphate generation, or cytosolic Ca^{2+} (in the de novo pathway, DAG may be derived directly from phosphatidic acid rather than from inositol phospholipids). To further test the possibility that DAG generation and protein kinase C activation may be important in the stimulation of glucose uptake, we examined the effects of another agonist, phenylephrine, which increases phospholipase C–mediated hydrolysis of inositol phospholipids in BC3H-1 myocytes[6] and would therefore be expected to increase DAG content and protein kinase C activity. In keeping with our postulate, phenylephrine was found to increase phosphoinositide turnover, protein kinase C activity, and 2-deoxyglucose (2-DOG) uptake.

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MATERIALS AND METHODS

Myocyte cultures. BC3H-1 myocytes were cultured in 35-mm dishes (unless indicated otherwise) in 3 ml of Dulbecco's minimal essential medium (DMEM) at 37°C under an air/CO_2 atmosphere, as described previously.[1,2,5] Cells were utilized 10–14 days after subculturing, when they had attained confluence and were maximally responsive to agonists.

Acute ^{32}P-labeling of phospholipids. Media were replaced with fresh media containing $^{32}PO_4$ (50 μCi/3 ml), and the cells were preincubated for 2 h. Agonists were then added and incubations were continued for the designated time periods. After incubation, the dishes were chilled, the media were aspirated, cold methanol was added, and the cells were dislodged by scraping. The cells and methanol were quantitatively transferred to extraction tubes containing chloroform and HCl (final volume ratios of $CHCl_3/CH_3OH/HCl$ = 2/1/0.075). Phospholipids were extracted, purified by thin-layer chromatography, localized by autoradiography, and counted for radioactivity, as described previously.[2,5]

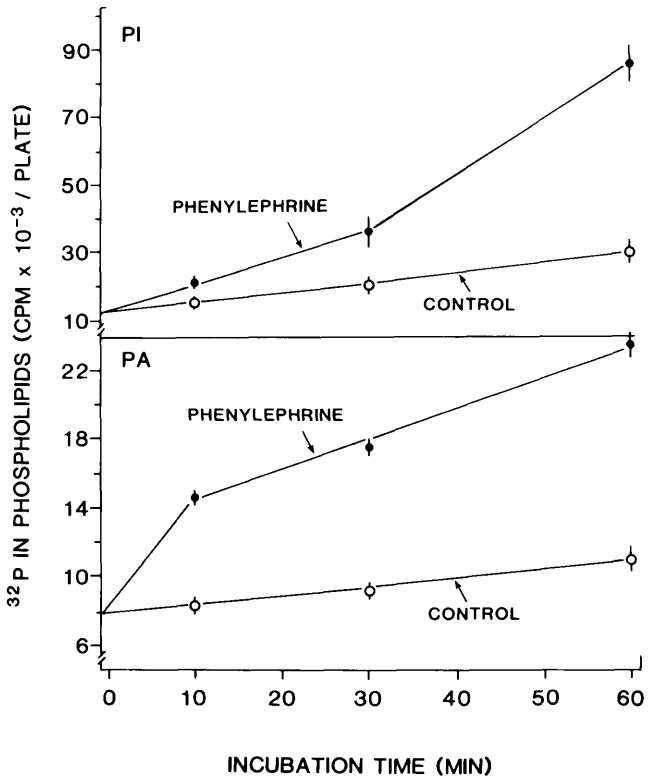

FIG. 1. Time course of 10^{-5} M phenylephrine effects on acute $^{32}PO_4$ labeling of phosphatidylinositol (PI) and phosphatidic acid (PA) in BC3H-1 myocytes. Mean values ± SE of 4 plates.

Chronic ^{32}P prelabeling of phospholipids. In addition to studying acute ^{32}P labeling of phospholipids (which would reflect changes in both de novo synthesis and degradative turnover and resynthesis), experiments were conducted to determine whether phenylephrine changed the inositol-phospholipid content of BC3H-1 myocytes. The experiments were identical to those conducted previously[2,5] in which insulin-induced increases in inositol-phospholipid content were demonstrable. To this end, the phospholipids were prelabeled by culturing for 3 days (10 μCi $^{32}PO_4$/3 ml medium) before the addition of agonist. Without changing the media, agonists were added for relatively short time periods, up to 60 min. Phospholipids were analyzed as described above. Under these conditions of prelabeling, phospholipids were labeled to constant specific activity, and consequent agonist-induced changes would reflect changes in phospholipid content rather than degradative turnover and resynthesis.

Inositol-phosphate determinations. Inositol phosphates were determined by Dowex-1 column chromatography, as described by Berridge and co-workers[7] and as employed by us previously in BC3H-1 myocytes.[5]

Cytosolic Ca^{2+} determinations. Details of this determination have been described previously.[5] In brief, BC3H-1 myocytes were loaded with 25 μM Quin 2/AM by incubation for 30 min at 37°C. The cells were dislodged, washed, resuspended in buffer containing 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM glucose, and 10 mM Hepes (pH 7.40), and then monitored continuously for fluorescence of the Quin 2–Ca^{2+} complex in a thermostated (37°C), continuously stirred cuvette, as described previously.[5] (Note: such resuspended myocytes manifest agonist-induced increases in 2-DOG uptake that are comparable with those observed in attached myocytes.[5])

Measurement of protein kinase C activity. Myocytes were rinsed and incubated in Dulbecco's phosphate-buffered saline (DPBS) containing 138 mM NaCl, 2.6 mM KCl, 1.5 mM KH_2PO_4, 0.5 mM $MgCl_2$, 8.1 mM Na_2HPO_4, 0.2 mM $CaCl_2$, and 0.1% bovine serum albumin (BSA) for 15 min at 37°C. Phenylephrine (10^{-5} M) was added as indicated, and incubation was continued for 30 min. The reaction was stopped by decanting media and rinsing twice with ice-cold DPBS. Cells were then kept at 0–4°C during all further processing. After scraping, the cell pellet was sonicated briefly in a solution containing 0.25 M sucrose, 20 mM Tris (pH 7.5), 2.5 mM $MgCl_2$, 1.2 mM EGTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 50 mM 2-mercaptoethanol. The sonicate was centrifuged at 105,000 × *g* for 30 min in a Beckman TL-100 ultracentrifuge. After removing the cytosol, the pellet was resuspended in a solution of 0.25 M sucrose, 20 mM Tris-HCl (pH 7.5), 10 mM EGTA, 2 mM EDTA, 1 mM PMSF, 50 mM 2-mercaptoethanol, and 0.1% Triton X-100 and incubated for 30 min on ice to solubilize protein kinase C. The resultant solubilized fraction was obtained by centrifugation at 105,000 × *g* for 30 min, and the supernatant was used as the "particulate or membrane fraction." Cytosolic fractions

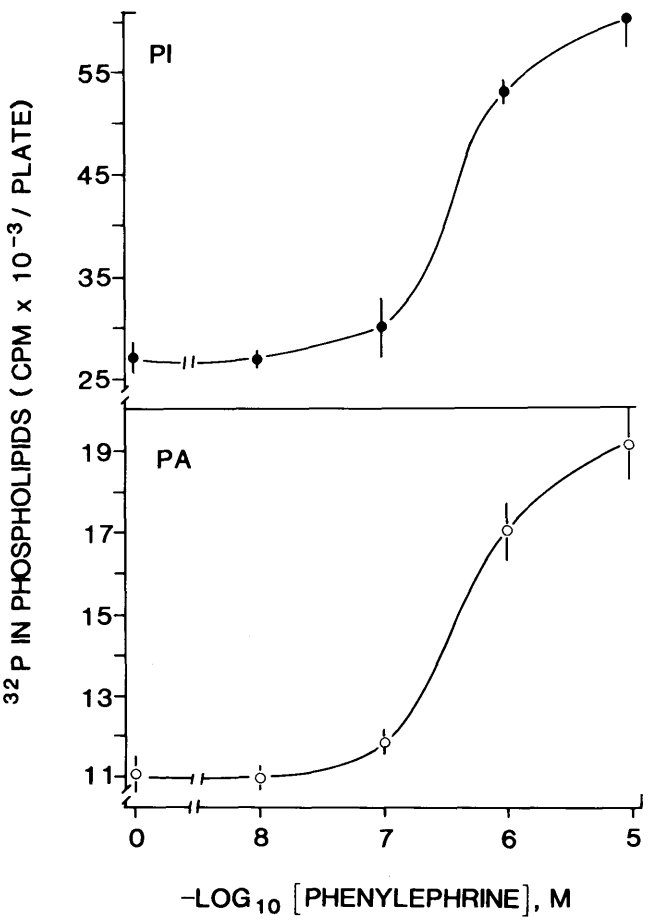

FIG. 2. Dose-related effects of phenylephrine on acute $^{32}PO_4$ labeling of phosphatidylinositol (PI) and phosphatidic acid (PA). Mean values ± SE of 4 plates. Incubation time was 60 min (after 2 h of preincubation with $^{32}PO_4$).

TABLE 1
Effects of phenylephrine and insulin on inositol-phosphate generation in BC3H-1 myocytes

Treatment	Inositol phosphates (cpm/dish)		
	Mono-PO_4	Di-PO_4	Tri-PO_4
None (control)	626 ± 41	24 ± 4	36 ± 3
Phenylephrine (10^{-5} M)	1262 ± 81 (<.001)	53 ± 6 (<.025)	66 ± 6 (<.025)
Insulin (200 nM)	627 ± 32 (NS)	17 ± 2 (NS)	29 ± 2 (NS)

Mature confluent myocytes in 60-mm dishes were prelabeled for 40 h with 8 μCi of [^{3}H]inositol in 8 ml of media. LiCl (10 mM) was added, and 15 min later treatments were added in small amounts of media (controls received media alone); incubation was then continued for another 30 min. After incubation, dishes were rapidly chilled, media were aspirated, and 2 ml of 10% trichloroacetic acid (TCA) were added. TCA extracts were chromatographed on Dowex-1 columns, and inositol (mono-, di-, and tri-)phosphates were eluted as described by Berridge et al.[7] Mean values ± SE of 3 determinations are shown. Similar results were observed in other experiments. *P* values (versus control; *t* test) are shown in parentheses. NS, not significant.

(5–15 μg) and particulate fractions (5–30 μg) were assayed in 250 μl of their respective buffers with 5 mM $MgCl_2$, 40 μg histone III-S, and 50 μM [γ^{32}-PO_4)ATP (1–5 × 10^5 cpm) in the presence of Ca^{2+} (0.5 mM in excess of EDTA/EGTA) and phosphatidylserine (PS) (5–10 μg) for protein kinase C and basal activity or 0.5 mM additional EGTA for basal activity alone. The assay reaction was carried out for 3 min at 30°C, and 2 ml of 25% (wt/vol) trichloroacetic acid was added to precipitate and extensively wash phosphorylated histone. Enzyme activity was linear with respect to incubation time and enzyme concentration. Protein kinase C activity was calculated by subtracting the basal kinase, or Ca^{2+}/PS–independent, activity (i.e., with EGTA) from the Ca^{2+}/PS–stimulated activity. Protein kinase M activity is reflected by the basal kinase activity (i.e., activity for histone phosphorylation in presence of EGTA, without Ca^{2+} or PS).

2-Deoxyglucose uptake. Uptake of [^{3}H]2-DOG was determined as described previously.[1,5,8] In brief, the myocytes were grown in 24-well cluster plates in media containing 25 mM glucose for 24–48 h before assay; rinsed three times with DPBS containing 138 mM NaCl, 2.6 mM KCl, 1.5 mM KH_2PO_4, 0.5 mM $MgCl_2$, 8.1 mM Na_2HPO_4, 0.2 mM $CaCl_2$, and 0.1% BSA; preincubated for 10 min in the same buffer; and then incubated for another 20 min with phenylephrine, TPA, and/or insulin, except in time-course experiments in which the treatment time was varied (note: rates of [^{3}H]2-DOG uptake in control cells did not change during the course of these incubations). [^{3}H]2-DOG (10 μM; 0.25 μCi) was added, and uptake was monitored over a 4-min period, as described previously.[1,5,8] As shown previously,[8] under conditions of this assay, [^{3}H]2-DOG uptake appears to primarily reflect transport rather than phosphorylation in the BC3H-1 myocyte. Phosphorylation of intracellular [^{3}H]2-DOG is nearly complete (~90%) during the first 5 min of assay, and the capacity of the phosphorylation system greatly exceeds that of the transport system. It is therefore unlikely that an increase in phosphorylation could be responsible for the increased rates of [^{3}H]2-DOG uptake presently observed with agonists. Moreover, stimulatory effects of agonists on the uptake of [^{3}H]2-DOG and 2-*O*-methylglucose (which is not phosphorylated) were shown to be similar in previous experiments.[8]

Materials. Phenylephrine, insulin, TPA, Quin 2/AM, and other biochemicals were purchased from Sigma (St. Louis, MO). Culture media were obtained from Grand Island Biological (Grand Island, NY). [^{3}H]2-DOG (sp act = 5 Ci/mmol) and carrier-free $H_3{}^{32}PO_4$ were obtained from New England Nuclear (Boston, MA). [^{3}H]inositol (sp act = 15 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO).

RESULTS

Effects of phenylephrine on acute ^{32}P labeling of phospholipids. As shown in Fig. 1, phenylephrine (10^{-5} M) provoked rapid increases in the ^{32}P labeling of phosphatidic acid (PA), and this was followed by increased labeling of phosphatidylinositol (PI). Phenylephrine did not influence the la-

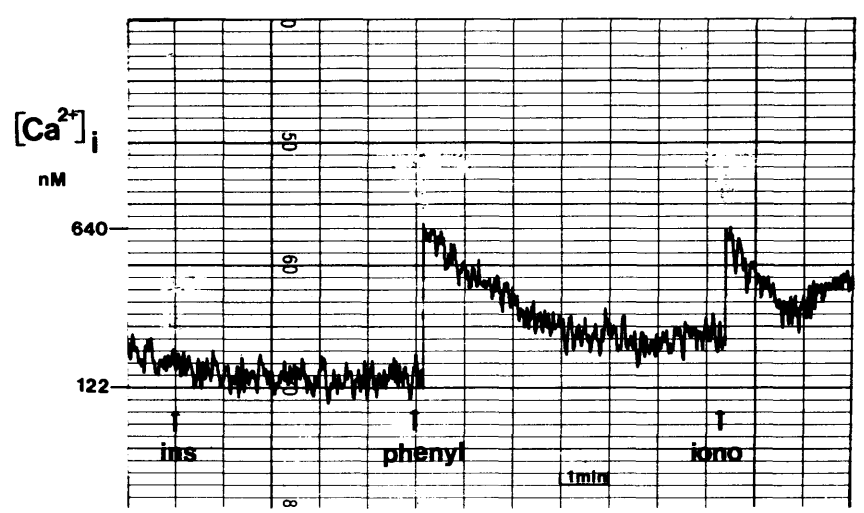

FIG. 3. Effects of 10^{-5} M phenylephrine (phenyl), 200 nM insulin (ins), and 500 nM ionomycin (iono) on cytosolic Ca^{2+} concentration in BC3H-1 myocytes. Representative experiment in which agents were added successively to same suspension of Quin 2–loaded myocytes is shown.

TABLE 2
Activation of membrane-bound protein kinase C by phenylephrine in BC3H-1 myocytes

Treatment	Kinase activity (pmol · min^{-1} · mg^{-1} protein)					
	Particulate			Cytosolic		
	Ca^{2+}-PS	EGTA	PKC	Ca^{2+}-PS	EGTA	PKC
None (control)	237 ± 4	8 ± 8	229	400 ± 47	61 ± 2	339
Phenylephrine (10^{-5} M)	381 ± 14	48 ± 6	333	297 ± 9	48 ± 6	249

Myocytes were treated for 30 min with or without phenylephrine, and kinase activity was determined in particulate (or membranous) and cytosolic fractions, in presence of Ca^{2+} and phosphotidylserine (PS) or EGTA, as described in MATERIALS AND METHODS. Protein kinase C (PKC) activity was determined by subtracting value with EGTA (protein kinase M or Ca^{2+}-PS–independent activity) from value with Ca^{2+} and PS. Means ± SE of 3 assays from representative experiment.

beling of other phospholipids, e.g., phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, and polyphosphoinositides (results not shown). Dose-related effects of phenylephrine on acute ^{32}P labeling of PA and PI are shown in Fig. 2; progressive increases were observed between 10^{-8} and 10^{-5} M phenylephrine.

Effects of phenylephrine on ^{32}P content of phospholipids after chronic ^{32}P prelabeling. After 3 days of culturing in the presence of $^{32}PO_4$, phospholipids had attained constant specific radioactivity. Acute addition of phenylephrine (10^{-5} M) to these prelabeled myocytes failed to change the ^{32}P labeling (i.e., content) of PA and PI (results not shown). This failure of phenylephrine to affect the contents of PA and PI contrasts with insulin-induced increases observed previously.[2,5]

Effects of phenylephrine on inositol-phosphate generation. As evidence for activation of phospholipase C in this study, we measured the generation of [^{3}H]inositol phosphates from inositol phospholipids prelabeled with *myo*-[^{3}H]inositol. As shown in Table 1, 10^{-5} M phenylephrine provoked twofold increases in inositol (mono-, di-, and tri-) phosphates. Insulin, on the other hand, did not increase inositol phosphates measurably. These effects are similar to those of phenylephrine[6] and insulin[5] observed previously. (In accordance with previous reports,[6] we were unable to observe consistent stimulatory effects of phenylephrine on inositol phosphate generation during the first 5 min of phenylephrine treatment. More time may be needed for measurable accumulation of the inositol phosphates.)

Effects of phenylephrine on cytosolic Ca^{2+}. As shown in Fig. 3, 10^{-5} M phenylephrine provoked an increase in cytosolic Ca^{2+}, as measured by Quin 2 fluorescence. Insulin, on the other hand, was without effect in the same myocyte preparations. In other experiments (not shown), dose-related effects of phenylephrine on cytosolic Ca^{2+} were observed, with progressive increases between 10^{-7} and 10^{-5} M phenylephrine.

Effects of phenylephrine on protein kinase C activity. The activation of protein kinase C by 10^{-5} M phenylephrine is shown in Table 2. As is apparent, membrane-bound Ca^{2+}/phospholipid–dependent kinase activity was increased by phenylephrine, and this was attended by a nearly stoichiometric decrease in corresponding cytosolic protein kinase C activity. [Presumably (see DISCUSSION), this apparent translocation is due to recruitment of the enzyme to membrane by diacylglycerol.] An increase in protein kinase M activity (i.e., basal kinase activity that persists in presence of 0.5 mM additional EGTA and in absence of Ca^{2+} and phosphatidylserine) was also apparent in the membrane fraction after phenylephrine treatment. (As discussed below, protein kinase M is considered to be a proteolytic product of activated protein kinase C.)

Effects of phenylephrine on 2-DOG uptake. As shown in Fig. 4, 10^{-5} M phenylephrine provoked a rapid increase in 2-DOG uptake, with maximal effects observed after 15–20 min. All subsequent experiments were conducted at 20 min of treatment. Dose-related effects of phenylephrine on 2-DOG uptake are shown in Fig. 5; maximal effects were observed at 10^{-5} M phenylephrine, and the ED_{50} was 0.6 μM.

Effects of phenylephrine on 2-DOG uptake were compared directly with those of insulin and TPA, which were described in greater detail previously.[1] As shown in Fig. 6, effects of 10^{-5} M phenylephrine and 200 nM insulin on 2-DOG uptake were essentially the same. TPA (500 nM, maximally effective concentration) was slightly more active than phenylephrine and insulin in this series of experiments and even more active in other experiments (see ref. 1). Addition of phenylephrine to cells maximally stimulated by TPA (500 nM) failed to in-

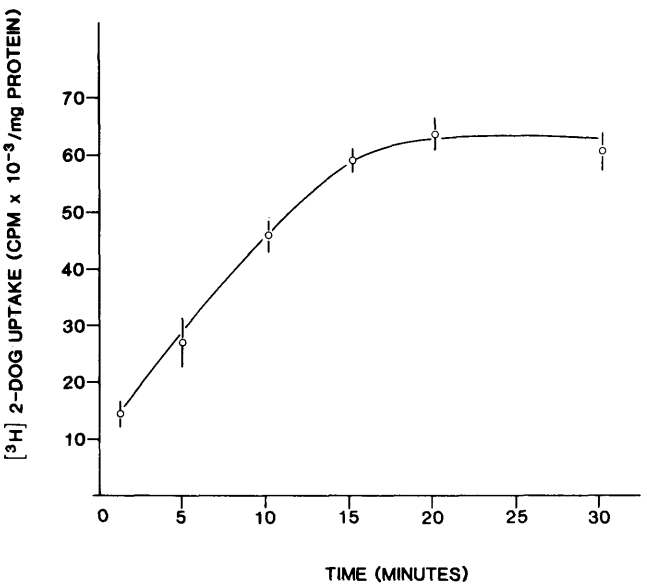

FIG. 4. Time course of 10^{-5} M phenylephrine on [^{3}H]2-deoxyglucose ([^{3}H]2-DOG) uptake in BC3H-1 myocytes. After preincubation for 15 min, phenylephrine was added and incubation was continued for indicated times. [^{3}H]2-DOG was then added and uptake was measured over 4-min period. Mean values ± SE of 6 determinations.

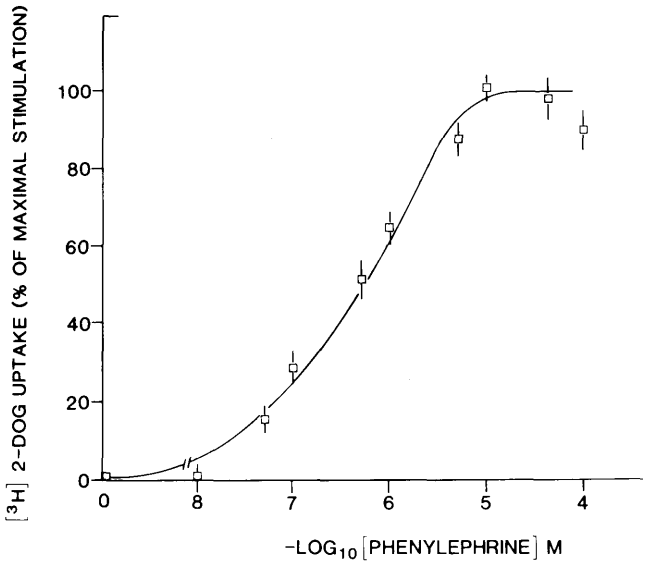

FIG. 5. Dose-related effects of phenylephrine on [^{3}H]2-deoxyglucose ([^{3}H]2-DOG) uptake in BC3H-1 myocytes. Cells were incubated for 20 min with indicated concentrations of phenylephrine and then assayed for [^{3}H]2-DOG uptake. Results are expressed as percent of maximal uptake induced by phenylephrine. Mean values ± SE of 6 determinations.

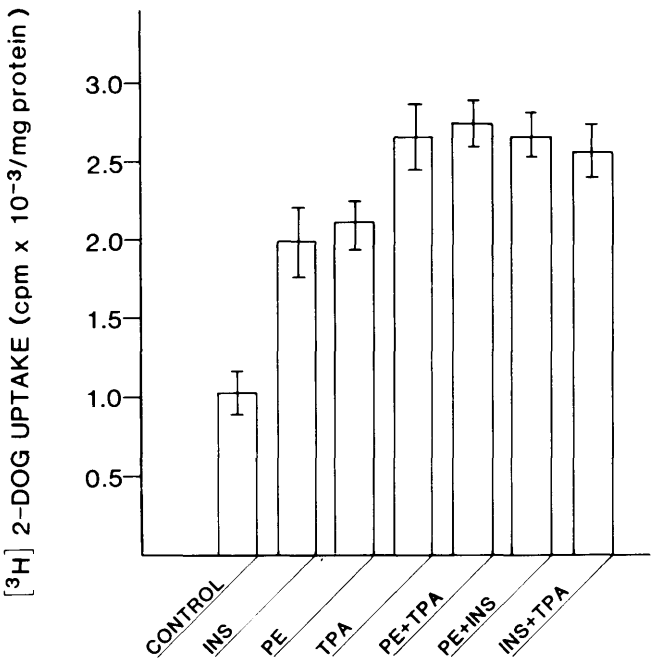

FIG. 6. Comparison of effects of 10^{-5} M phenylephrine (PE), 200 nM insulin (INS), and 500 nM TPA on [^{3}H]2-deoxyglucose ([^{3}H]2-DOG) uptake in BC3H-1 myocytes. Cells were incubated for 20 min with indicated agents and then assayed for [^{3}H]2-DOG uptake. Mean values ± SE of 5 determinations.

crease 2-DOG uptake further. Similarly, addition of insulin (200 nM) to maximally stimulated cells (by 500 nM TPA) also failed to increase 2-DOG uptake further. However, the combination of phenylephrine and insulin were regularly additive, and together they increased 2-DOG uptake to a level comparable to that observed with TPA.

Because phenylephrine may increase 2-DOG uptake by changes in Ca^{2+} as well as by DAG-induced protein kinase C activation, experiments were conducted to assess the importance of Ca^{2+} in the 2-DOG response. As shown in Table 3, incubation in nominally Ca^{2+}-free media containing 1 mM EGTA caused basal 2-DOG uptake to increase slightly (also noted previously[5]). More important, the phenylephrine effect on 2-DOG uptake was not diminished by conditions of extracellular Ca^{2+} deficiency.

DISCUSSION

Like insulin, phenylephrine was found to stimulate phosphoinositide metabolism in BC3H-1 myocytes. However, unlike insulin, which seems to primarily increase de novo synthesis (and contents) of PA and inositol phospholipids in BC3H-1 myocytes,[2,5] it appeared that phenylephrine primarily stimulated phospholipase C–mediated hydrolysis of inositol phospholipids, as evidenced by increases in inositol phosphates and increases in $^{32}PO_4$ incorporation into PA and PI in the absence of a concomitant increase in PI content. This effect of phenylephrine on phospholipid metabolism in BC3H-1 myocytes is similar to those observed with α_1-adrenergic agents in other tissues.[9–11]

Phenylephrine-induced activation of phospholipase C in these myocytes resulted in increased generation of inositol phosphates and activation of protein kinase C. Conceivably,[12–15] the increases in inositol triphosphate (IP_3) may have been responsible for the observed increases in cytosolic Ca^{2+}, but this is not certain because measurable increases in IP_3 were only evident after 5 min of phenylephrine treatment. Although changes in DAG content were not directly measured in this study, the observed increases in inositolphosphates and particulate or membrane-bound protein kinase C activity indicate that DAG was also increased by phenylephrine.[3,12–14]

The phenylephrine-induced increase in membrane-bound protein kinase C activity observed here is similar to that observed with gonadotropin-releasing hormone in pituitary gonadotrophs.[15] Apparently there was a stoichiometric translocation of protein kinase C from the cytosol to the membraneous fraction, presumably resulting from an increase in

TABLE 3
Failure of Ca^{2+} deficiency to influence phenylephrine-induced increases in 2-deoxyglucose uptake

Medium			
[$CaCl_2$] (mM)	[EGTA] (mM)	[Phenylephrine] (μM)	[^{3}H]2-deoxyglucose uptake (cpm/mg protein)
0.9	0	0	10202 ± 1116
0.9	0	50	30305 ± 5636 ($P < .01$)
0	1	0	16813 ± 1416
0	1	50	34160 ± 3137 ($P < .001$)

[^{3}H]2-deoxyglucose ([^{3}H]2-DOG) uptake was determined as described in MATERIALS AND METHODS, except that after 24 h of incubation in 25 mM glucose, myocytes were rinsed 3 times and incubated for another 60 min in Ca^{2+}-free Dulbecco's phosphate-buffered solution containing 25 mM glucose, 0.1% bovine serum albumin, and 1 mM EGTA, then rinsed 3 times and incubated for 20 min with or without phenylephrine in same buffer, which had no glucose present. [^{3}H]2-DOG was added and uptake was measured. Mean ± SE of 4 determinations. *P* values (phenylephrine versus corresponding control) were determined by *t* test.

DAG, which is generated within cellular membranes and binds protein kinase C. The resulting interaction of membrane-bound DAG and protein kinase C activates the latter by decreasing the Ca^{2+} concentration required for protein kinase C activation.[3] Activated protein kinase C in turn apparently may be proteolytically converted to protein kinase M, which no longer requires Ca^{2+} and lipids for activation.[16] Our demonstration of phenylephrine-induced increases in membrane-bound protein kinases C and M, coupled with increased PI turnover (as evidenced by increases in acute $^{32}PO_4$ incorporation into PA and PI and increases in inositol phosphate generation), provides convincing evidence that phenylephrine activates phospholipase C and the DAG–protein kinase C intracellular signaling system in BC3H-1 myocytes.

The insulin-like effects of phenylephrine on 2-DOG uptake are of considerable interest. Maximally effective concentrations of phenylephrine and insulin provoked nearly identical increases in 2-DOG uptake. In addition, the time course for 2-DOG uptake observed presently for phenylephrine (15–20 min to provoke maximal effect) is similar to that observed previously with insulin treatment.[1,8] Apparently, neither agent alone maximally activated 2-DOG uptake, and further stimulation could be provoked by adding the other agent or TPA (see ref. 1). However, in the presence of maximally effective concentrations of TPA (and presumably maximal activation of protein kinase C), neither phenylephrine nor insulin provoked further increases in 2-DOG uptake. These results suggest that phenylephrine, insulin, and TPA may stimulate 2-DOG uptake by a common final effector mechanism. Because both phenylephrine and insulin increase DAG (apparently by different phospholipid mechanisms), both agonists activate protein kinase C, and TPA acts as an analogue of DAG to activate protein kinase C,[4] it is plausible to suggest that this common final effector mechanism for 2-DOG uptake involves protein kinase C activation. Interestingly the glucose transporter itself has been shown to be phosphorylated by protein kinase C,[17] and this may be important for the activation or translocation of the glucose transporter to the plasma membrane.[18,19]

The fact that effects of phenylephrine, insulin,[5] and TPA[5] on 2-DOG uptake are not compromised appreciably by incubation of myocytes in nominally Ca^{2+}-free medium containing 1 mM EGTA suggests that DAG-induced protein kinase C activation is of greater importance than changes in Ca^{2+} (which a priori are expected to be blunted by EGTA) in the action of these agents on 2-DOG uptake. This finding, however, must be interpreted cautiously and, moreover, does not rule out a role for intracellular Ca^{2+}, which is also required, at least permissively, in the activation of protein kinase C by DAG.[3]

Increases in 2-DOG or 3-*O*-methylglucose uptake have also been observed in the actions of epidermal growth factor[20] and pancreatic secretagogues, carbachol and cholecystokinin.[21] In addition, increases in glucose metabolism are evident in the actions of thyroid-stimulating hormone in the thyroid,[22] adrenocorticotropin in the adrenal gland,[23] and a variety of substances (insulin, oxytocin, thyroid-stimulating hormone, adrenocorticotropin, epinephrine, phospholipase C, and TPA) in adipose tissue.[24–27] One effect that seems to be common in the action of all or most of these agents is increased generation of DAG, either via increased inositol phospholipid hydrolysis or increased de novo phospholipid synthesis (note: PA can be directly converted to DAG) and consequent activation of protein kinase C. On the whole, these reports and our findings are compatible with our hypothesis that DAG-induced activation of protein kinase C may be important for increasing glucose uptake and metabolism in the target tissues of various agonists that influence phospholipid metabolism. Obviously, further work is needed to test this hypothesis.

ACKNOWLEDGMENTS

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