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INSULIN AND GLUCOSE MODULATE PROTEIN KINASE C ACTIVITY IN RAT ADIPOCYTES

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In the presence of 1 mM glucose, insulin (10 ng/ml) increases both catalytic and receptor-binding properties of adipocyte cytosolic protein kinase C (PKC). Preincubation of adipocytes with 10 mM glucose raises basal PKC catalytic activity and prevents further stimulation of this enzyme by insulin. The effect of hyperglycemia is likely to be mediated by direct conversion of glucose into diacylglycerol. Thus, an incorporation of ^{14}C-glucose into diacylglycerol is enhanced 10-fold in the presence of 10 mM glucose. These observations indicate that, in normal adipocytes, both insulin and glucose activate PKC; hyperglycemia eliminates the ability of insulin to stimulate this enzyme, thereby interfering with insulin action.

Recent studies of Bollag, et. al. (1) demonstrated that protein kinase C (PKC) directly phosphorylates the insulin receptor and reduces receptor protein-tyrosine kinase (TK) activity by 65%. Takayama, et. al. (2) have observed that activation of PKC by phorbol ester resulted in stimulated phosphorylation of serine residues and a 50% reduction in insulin-stimulated tyrosine phosphorylation. These receptors also exhibited a reduced TK activity *in vivo* and *in vitro*. Since activation of insulin receptor involves TK as one of the initial steps in transduction of insulin-generated signals (3), reduction in TK activity may result in decreased magnitude of insulin action. If insulin is capable of activating PKC in its target tissues, then PKC effect on TK activity of insulin receptor may provide for an elegant feedback system diminishing or even terminating insulin action.

In this study, the effect of insulin on PKC in normal rat adipocytes was examined. We also investigated the possibility

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that hyperglycemia may modulate the effect of insulin on PKC activation.

METHODS

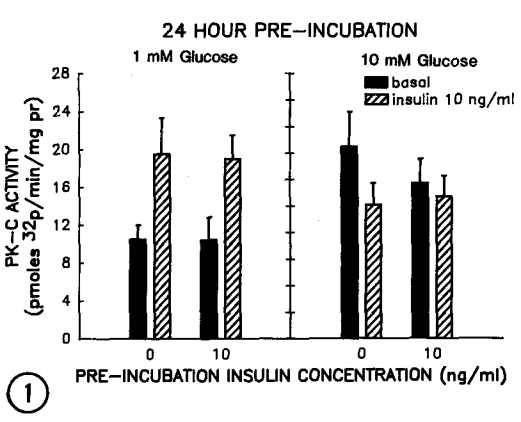
Isolated rat adipocytes were preincubated for 24 hrs with 1 or 10 mM glucose in the presence or in the absence of insulin (10 ng/ml). After the preincubation, the cells were washed three times and incubated for 40 min in glucose- and insulin-free medium to eliminate any possible influence of high glucose or insulin present during 24 hr preincubation (4). The cells were resuspended in Krebs-Ringer bicarbonate buffer and one half of the cells was then exposed to 10 ng/ml of insulin for 20 min at 37°C (insulin-stimulated PKC activity), while the remaining cells were incubated without insulin (basal PKC activity). At the end of the incubation, the cells were washed and sonicated for differential centrifugation (5). Plasma membrane and cytosolic fractions were isolated and used immediately in histone III-S phosphorylation assay to estimate PKC activity (6,7). The activity was determined by the difference in the pmoles of ^{32}P incorporation per mg protein per min in the presence of Ca^{+} (1 mM), diolein (8 μM), and phosphatidylserine (60 μM), and in the absence of these agents (but in the presence of 1 mM EGTA).

^{3}H-PDB (New England Nuclear, Boston, MA) binding to cytosolic and membrane-associated PKC was carried out as described by Uchida, et. al. (8) and Cambier, et. al. (9). The cells were isolated and preincubated for 24 hr as described above, except that insulin was absent from preincubation media. ^{3}H-PDB binding was performed using 20 nM ^{3}H-PDB as a ligand, 1.5 μM 4β-phorbol 12 β myristate 13 α acetate (TPA) to assess non-specific binding, and filtration through 2.4 cm Whatman GF/C glass filter to remove any unbound ligand. Both basal and insulin-stimulated ^{3}H-PDB binding were determined.

Determinations of [U^{14}C]-glucose (sp. activity 304.7 μCi/μMole) incorporation into diacylglycerol in isolated adipocytes was performed as described by Tamarit-Rodriguez, et. al. (10), using thin layer chromatography. The position of DAG was detected under the UV light after spraying the plates with 2',7'-dichlorofluoresclin. The spots corresponding to DAG markers were scraped into scintillation vials and counted for radioactivity present.

RESULTS AND DISCUSSION

In adipocytes pre-incubated with 1 mM glucose, insulin enhanced both cytosolic PKC catalytic activity (Fig 1) and ^{3}H-PDB binding (Fig 2). The magnitude of insulin action was approximately the same in both assays, suggesting that enhancement of PKC activity may be secondary to an increase in enzyme concentration. The origin of the PKC contributing to the increase in ^{3}H-PDB binding is unknown. Recently, Pershadsingh, et. al. (11) have reported an insulin-induced decrease (27%) in ^{3}H-PBu_2 (phorbol 12,13 dibutyrate) binding to



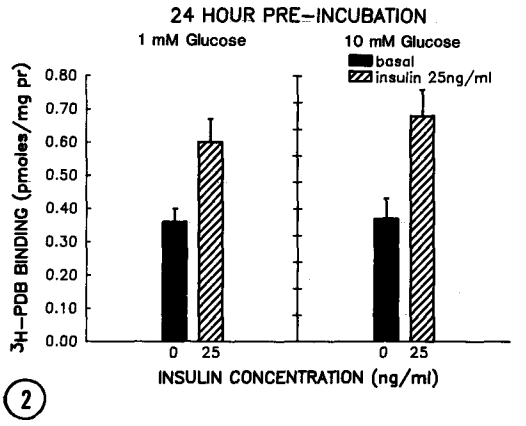


Figure 1: Effect of glucose and insulin on cytosolic PKC activity in rat adipocytes. Results represent mean ± SEM of 5 experiments, each performed in duplicate.

Figure 2: The effect of glucose and insulin on ^{3}H-PDB binding to cytosolic PKC in rat adipocytes. Results represent specific ^{3}H-PDB binding expressed as mean ± SEM of 3 experiments, each performed in triplicate. Insulin-stimulated ^{3}H-PDB binding was significantly ($p < 0.01$) greater than basal under both conditions (1 and 10 mM glucose). Results of ^{3}H-PDB binding to plasma membrane fractions are reported in the text.

the adipocyte PM, which could partially account for a 42% increase observed in the cytosol. In the present experiments, insulin induced a 20% decrease in ^{3}H-PDB binding to the PM and a 65% increase in cytosolic binding. It is conceivable that PKC from other cellular organelles was translocated to the cytosol.

We did not observe insulin-induced changes in PKC activity (determined by HP assay) in the PM fraction (data not shown). This is in agreement with several previous observations (5,12-14), indicating that insulin does not cause translocation of PKC to the plasma membrane. The presence of 10 ng/ml insulin in the 24 hr preincubation medium (1 mM glucose) did not alter either basal or subsequent insulin-stimulated PKC activity (Fig 1).

In contrast, preincubation of adipocytes with 10 mM glucose significantly effected basal PKC activity and the response to insulin. Basal activity of cytosolic PKC was significantly increased ($p < 0.05$) regardless of whether or not insulin was present in the culturing media (Fig 1). Despite this augmentation in basal PKC catalytic activity (HP assay), the basal concentration of the enzymes was unchanged (PDB binding)

Table 1. Effect of 10 mM glucose on cytosolic PKC activity in normal adipocytes

	PKC activity pmoles ^{32}P/min/mg protein	P
1 mM Glucose		
a) alone	11.08 ± 1.05	
b) + insulin (10 ng/ml)	7.34 ± 2.77	< .05 vs. a
10 mM Glucose		
c) 2 min (alone)	9.74 ± 2.11	NS vs. a
d) 2 min (+ insulin 10 ng/ml)	28.86 ± 7.02	< .05 vs. c
e) 2 hrs (alone)	20.80 ± 4.47	< .01 vs. a
f) 2 hrs (+ insulin 10 ng/ml)	21.27 ± 5.28	NS vs. e
g) 24 hrs (alone)	21.25 ± 2.56	NS vs. e
h) 24 hrs (+ insulin 10 ng/ml)	15.20 ± 1.54	NS vs. g

Adipocytes were preincubated with 10 mM glucose for 2 min, 2 or 24 hrs. PKC activity was determined by HP assay as described in Figure 1 legend. The results represent mean ± SEM of 3 to 5 experiments, each conducted in duplicate or triplicate.

and remained similar to that observed in adipocytes cultured with 1 mM glucose. Twenty-four hour preincubation with 10 mM glucose completely abolished insulin-induced increases in PKC catalytic activity (Fig 1) without altering insulin-stimulated increase in PKC concentrations (Fig 2). Finally, the presence of insulin in the 24 hour preincubation medium did not alter the response to acute addition of insulin (Fig 1). The activity of PKC in the PM fraction remained unchanged in all experiments (not shown).

These observations suggest that glucose (10 mM) stimulates selectively the catalytic subunit of PKC. Under these circumstances, insulin continues to increase the amount of binding subunit, but provides no further stimulation of the catalytic subunit. This effect of 10 mM glucose was evident after 2 hrs of incubation but not after 2 min (Table 1).

The mechanism whereby glucose activates the catalytic activity of PKC is unknown. Glucose-induced increases in $[Ca^{2+}]i$ and in diacylglycerol (DAG) production are among the possibilities. To examine the first possibility, we preincubated adipocytes as described above in the presence of

Table 2. [U ^{14}C] Glucose Incorporation into Diacylglycerol in Isolated Adipocytes*

Time	with 1 mM glucose (pmoles/mg protein)	with 10 mM glucose (pmoles/mg protein)
4 min (n=4)	41.33 ± 3.06	385.95 ± 39.03
20 min (n=6)	46.30 ± 5.43	348.57 ± 38.45
2 hr (n=6)	37.44 ± 5.47	352.93 ± 40.69

*[U ^{14}C] glucose specific activity 304.7 μCi/μMole incorporation into DAG was assessed by thin layer chromatography as described by Tamarit-Rodriguez, et. al. (10).

the Ca^{2+} channel blocker, verapamil (30 μM). In adipocytes preincubated with 10 mM glucose, verapamil reduced cytosolic free calcium concentration from 162 ± 11 to 99 ± 13 nM, $p < 0.05$ (measured with fura-2 as previously described, 15). However, verapamil failed to affect the augmentation of PKC activity induced by 10 mM glucose (18 ± 3 pmoles/^{32}P/min/mg protein vs. 21.2 ± 3 with 10 mM glucose alone). This suggested that the effect of glucose on PKC activity was not calcium mediated.

We then addressed the possibility of glucose being converted into DAG and the rate of such conversion being increased in the presence of high glucose concentrations. The higher rate of ^{14}C-glucose incorporation into DAG reflects increased DAG production, but does not indicate the source of newly formed DAG. DAG can be derived in the process of triphosphoinositide hydrolysis or as a result of de-novo synthesis, largely from glycerol-3-phosphate via phosphatidic acid (see 16 for review).

The effect of glucose concentration on [U ^{14}C]-glucose carbon incorporation into DAG was examined at 4 and 20 min of incubation and at 2 hrs (Table 2). In the presence of 10 mM glucose, there was 8 to 10-fold increase in amount of ^{14}C-glucose incorporation into DAG at 4, 20 min and 2 hr of incubation. Although these data do not directly distinguish between the sources of DAG, they indicate that in the presence of hyperglycemia, increasing amounts of glucose are converted into DAG and may thereby activate PKC. Since insulin has been shown to rapidly activate de novo DAG synthesis (17), the process of DAG formation is likely to represent the point of convergence of insulin and glucose influence on signal transduction system of insulin target cells.

Taking these observations in conjunction with the ability of PKC to reduce TK activity of insulin receptors (1,2), one may postulate that activation of PKC by hyperglycemia may result in diminution of insulin receptor TK activity and impaired insulin action. Furthermore, activation of PKC may, at least in part, explain post-receptor defect(s) in insulin target tissues induced by hyperglycemia and/or hyperinsulinemia.

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