Insulin-Like, Antilipolytic Effect of 12-O-Tetradecanoyl Phorbol 13-Acetate in Rat White Adipocytes (43245)

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Abstract. **Previous experimental data documenting an insulin like-effect of 12-O-tetradecanoyl phorbol 13-acetate (TPA), a specific activator of protein kinase C, on glucose transport in adipocytes prompted us to test the hypothesis that TPA might display another insulin-like effect, i.e., antagonize catecholamine-induced lipolysis. TPA (100 n*M*) led to a decrease of both free fatty acid (41%) and glycerol (58%) release due to 1 μM norepinephrine stimulation in isolated rat adipocytes. TPA also diminished the antilipolytic effect of insulin (5 $ng \cdot ml^{-1}$) in the presence of 1 μM norepinephrine. Thus, the residual lipolysis with insulin was 25% for free fatty acids and 24% for glycerol release. In the presence of TPA, these values increased to 50% and 45%, respectively. TPA (100 n*M*) addition to isolated adipocytes induced protein kinase C translocation from the cytosol to the membrane fraction. In control cells, 94.7 ± 2.9% of the enzyme was found in the cytosol, with the rest found in the membrane. At 10 min after TPA (100 n*M*) addition, the corresponding value was 43.6 ± 17.4%, with the rest in the membrane ($n = 6$, $P < 0.05$). These findings indicate that protein kinase C might be involved not only in the insulin action on glucose transport, but also in the mechanism of insulin's antilipolytic action.** [P.S.E.B.M. 1991, Vol 197]

Experimental evidence has accumulated in the last several years showing that phorbol esters, mainly 12-O-tetradecanoyl phorbol 13-acetate (TPA), display an array of insulin-like effects on adipocyte metabolism. Essentially, these consist in stimulation of glucose transport and oxidation (1, 2), increase in lipogenesis when the process is limited by glucose transport (3), and translocation of protein kinase C (PKC) from the soluble, cytosolic fraction to the membrane fraction (1, 2). On the basis of these and other data (for a review, see reference 4), the general consensus has emerged that PKC might mediate, in part, insulin effects on glucose transport and metabolism in adipocytes. It is classically known that besides its effect on adipocyte metabolism related to glucose transport, insulin antagonizes catecholamine-induced lipolysis. The mechanism of such an action consists of inactivation of the hormone-sensitive lipase (5, 6) through enzyme dephosphorylation (7).

While investigating possible effects of *Escherichia coli* endotoxin (ET) on PKC activity and lipogenesis in rat adipose tissue, we found that TPA mimics the antilipolytic effect of insulin and that such an effect is associated with PKC translocation from the cytosolic, soluble fraction to the membrane fraction. In this study, we present such experimental data and discuss their possible implication for PKC involvement in the mechanism of action of insulin.

Materials and Methods

Animals. Male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA), weighing 280–370 g, having free access to food and water, were used. Because these animals served as controls to ET-treated rats, they received sterile saline through a jugular vein catheter, implanted the day before the experiment, at a rate of 1 $ml \cdot h^{-1}$, for 3 hr. During the infusion, the animals were conscious and unrestrained. At the end of infusion, the animals were injected intraperitoneally with Nembutal (Abbot Laboratories, North Chicago, IL) (5 mg/100 g body wt) and, while under anesthesia,

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the epididymal fat pads were removed and used for adipocyte isolation (8).

Measurement of Lipolysis. Cells ($7–12 \times 10^4$ ml^{-1}) were incubated in a final volume of 2 ml of Krebs-Ringer bicarbonate (5 m*M*)-Hepes (20 m*M*) buffer (containing 1% extensively dialyzed fatty acid-free bovine serum albumin), pH 7.45, at 37°C. TPA or the carrier (dimethyl sulfoxide, 0.25% final concentration was added to the corresponding tubes at the beginning of the incubation, followed, 30 min later, by addition of other ingredients. Incubation continued for 1 hr. The reaction was stopped by chilling the tubes in an ice bath. Subsequently, free fatty acids and glycerol were measured in the medium, and triglyceride content was measured in the cells, by enzymatic procedures (9–11). The quantitation of data has been described previously (12).

Measurement of Protein Kinase C Activity and Translocation. Cells (0.5-ml packed cell volume) were incubated for 10 min at 37°C, in a final volume of 4 ml in the presence of TPA (100 n*M*, final concentration) or carrier (dimethyl sulfoxide, 0.25% final concentration). At the end of incubation, the cells were centrifuged and the infranatant was removed by suction. The cells were treated with 1 ml of a mixture containing (m*M*, final concentration): 3-(*N*-morpholino)propanesulfonic acid, 17; sucrose, 250; EDTA, 2.5; phenylmethylsulfonylfluoride, 1; dithiothreitol, 5; leupeptin, 10 $\mu g \cdot ml^{-1}$; and digitonin, 0.8 $mg \cdot ml^{-1}$ (pH 7.20). After vigorous mixing, the tubes were placed in an ice bath for 5 min and centrifuged for 20 min at 48,000*g* at 4°C. After removal of the floating fat cake, the supernatant, referred to here as cytosolic or soluble fraction, was used for partial PKC purification. The remaining pellet was treated with 0.45 ml of the digitonin containing medium and 0.05 ml of Triton X-100 (1%, w/v). After vigorous mixing, the mixture was sonicated for 1 min (W-375; Heat Systems-Ultrasonics, Inc., Plainview, NY) at the maximal setting for the microtip. After 1 hr on ice, the tubes were centrifuged as described above and the supernatant was used for partial purification of the enzyme. This fraction is referred to here as membrane or insoluble fraction. The partial purification of the enzyme was performed according to the method of Farrar and Anderson (13) using DE-52 cellulose (Whatman LabSales, Inc., Hillsboro, OR). PKC assay in purified extracts was performed according to the method of Kuo *et al.* (14). The enzyme activity was related to the total amount of protein assayed (15) in both fractions before partial enzyme purification.

Chemicals. TPA, norepinephrine (bitartrate), histone S-III, and the kit for triglyceride enzymatic assay were from Sigma Chemical Co. (St. Louis, MO). [γ-^{32}P]ATP (25 $Ci \cdot mmol^{-1}$) was from ICN (Irvine, CA); enzymatic kits for free fatty acid and glycerol assay were from Boehringer Mannheim Biochemicals (Indianapolis, IN); insulin was from CalBiochem Corp. (La Jolla, CA). All other chemicals were of the highest purity commercially available.

Statistical analysis of the data was done using Student's *t* test.

Results

The data presented in Figure 1 show that TPA considerably diminished the norepinephrine lipolytic effect, as reflected by the release of both free fatty acids (29%) and glycerol (37%). Additionally, TPA antagonized the insulin's antilipolytic potency. At the concentration used, i.e., 5 $ng \cdot ml^{-1}$, insulin decreased by 75 and 76% the lipolytic response to norepinephrine in terms of both free fatty acids and glycerol release, respectively. In the presence of TPA, the corresponding values for insulin inhibition were only 50 and 55% (Fig. 1). Finally, TPA, at the concentration identical to the one giving the antilipolytic effects, i.e., 100 n*M*, produced significant displacement of PKC from the soluble to the membrane fraction of adipocytes (Fig. 2). Thus, of a total PKC activity corresponding to 37.6 ± 2.9 $pmol \cdot min^{-1} \cdot mg^{-1}$ protein (n = 7), 94.7 ± 2.9% was found in the cytosol, with the rest being located in the pellet fraction. After incubation with TPA, these values

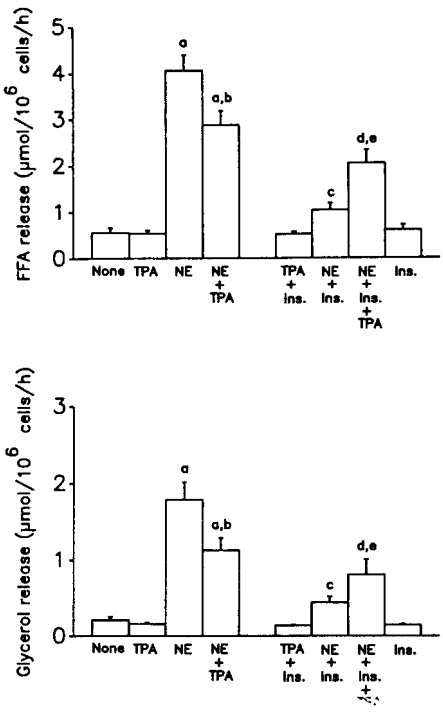

Figure 1. Lipolysis-antilipolysis patterns in isolated rat adipocytes as reflected by free fatty acids (FFA) (upper panel) and glycerol (lower panel) release. The following ingredient concentrations were used: TPA, 100 n*M*; norepinephrine (NE), 1 μM; insulin (Ins), 5 $ng \cdot ml^{-1}$. The columns are mean ± SE (vertical bars) for five to six individuals in a group. The following statistically significant (at $P < 0.05$ or smaller) differences apply: a, vs none and TPA; b, vs NE; c, vs NE and NE + TPA + Ins; d, vs NE + TPA and NE + Ins; e, vs Ins.

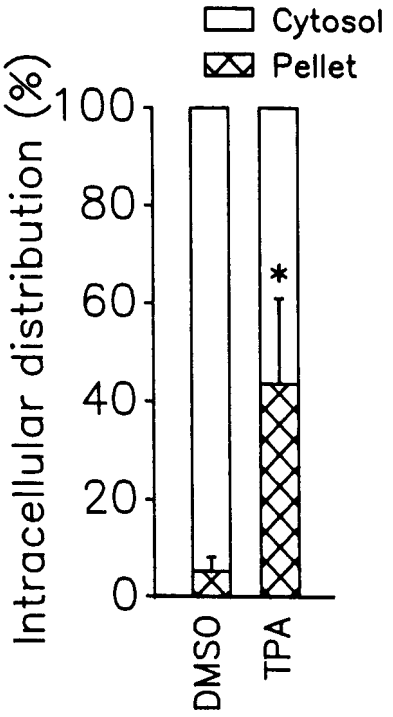

Figure 2. Effect of TPA (100 n*M*) on intracellular distribution of PKC activity in isolated rat adipocytes. The asterisk shows a statistically significant difference between TPA- and dimethyl sulfoxide (DMSO)-treated cells ($n = 4$, $P < 0.05$). The total PKC activity was 37.6 ± 2.9 pmol·min^{-1} mg^{-1} protein ($n = 7$).

were 56.4 ± 17.4% for the cytosol and 43.6 ± 17.4% for the membrane fraction ($n = 4$, $P < 0.05$, Fig. 2).

Discussion

The experimental data presented in this study demonstrate that the insulin-like effects of TPA, a potent activator of PKC, are not restricted to glucose transport, as previously documented (4), but extend also to antilipolysis. Our experimental data confirm the findings of Solomon and Palazzolo (16), who reported an antilipolytic effect of TPA in the presence of epinephrine in rat white adipocytes. Our experiments were designed to demonstrate that such an effect is concomitant with PKC translocation from the soluble to the membrane fraction of adipocytes. In contrast to the work of Solomon and Palazzolo (16), who found that TPA itself has a mild lipolytic effect, we were not able to detect such an effect of TPA, in spite of the fact that the concentration used in our work (100 n*M*) was the same as that in the cited study. One should consider at least two factors that might potentially account for this discrepancy. Whereas Solomon and Palazzolo (16) used Holtzman strain rats, weighing 175–250 g, in their lipolysis studies, we used Sprague-Dawley rats, weighing 280–370 g. The other factor is the difference in the basal lipolytic rates reported in the two studies. In the cited study, the value for this parameter was 0.03 μmol of glycerol released/10^6 cells/hr and increased to 0.10, same units, in the presence of 100 n*M* TPA. In our experiments, the basal lipolytic rate was much higher, i.e., 0.21 μmol of glycerol released/10^6 cells/hr, and remained essentially unaltered by 100 n*M* TPA. It is not excluded that higher basal lipolytic rates, as in our study, may mask a potential lipolytic effect of TPA.

In addition, in our study a similarity can be established between TPA effects on glucose transport and on catecholamine-induced lipolysis. It has been shown that at insulin concentrations maximally stimulating glucose transport, TPA antagonized insulin's effect by about 20% (17). Such an antagonizing effect of TPA was also observed in this study with respect to the antilipolytic action of insulin, although quantitatively it was more pronounced than the one seen on glucose transport. The antagonizing action of TPA on maximal insulin-stimulated glucose transport in rat adipocytes was explained on the basis of TPA's ability to induce an increase in the K_m for ATP of the insulin receptor kinase, rather than on its ability to alter the insulin-receptor interaction (17). Whether insulin acts as a glucose transport enhancer or as an antilipolytic agent, a necessary step in its action is the stimulation of autophosphorylation of the receptor kinase. Therefore, a change in the affinity for ATP of the insulin receptor kinase should modulate insulin's effect on both glucose transport and lipolysis. This is very likely to be the common denominator for TPA antagonizing effects of maximal insulin action on both glucose transport and lipolysis.

The mechanism by which TPA mimics insulin effects on adipocyte metabolism is believed to consist in PKC activation associated with enzyme translocation from the cytosol to the membrane (1, 2). Previous work demonstrated that insulin induces the translocation of PKC from the cytosol to the plasma membrane (18) and an increase in the activity of the enzyme associated with the plasma membrane (19). Experimental data were also presented (20) suggesting that PKC might participate in the translocation of glucose carriers from intracellular sites to the plasma membrane. Overall, however, the mechanism of PKC participation in insulin action is not well understood. This issue was recently reviewed by Klip and Douen (4). If the only mechanism of action of TPA in white adipocytes consists in PKC activation, then it seems reasonable to assume that both insulin-mimicking effects of TPA, i.e., on glucose transport and catecholamine-induced lipolysis, are solely mediated by PKC activation. It should be emphasized, however, that this does not imply that PKC is the sole modulator of the mechanism of action of insulin.

The following hypothesis can be advanced at present. The activation-inactivation of hormone-sensitive lipase is effected through phosphorylation-dephosphorylation reactions (6). Insulin has been shown to de-

crease the phosphorylation state of the hormone-sensitive lipase through two mechanisms: one linked to cAMP participation and the other independent of cAMP (7). The latter might involve PKC participation. TPA might act through a similar mechanism if, by activating PKC, it would promote enhanced dephosphorylation of the lipase. This could be achieved, for instance, by activating a phosphatase through a phosphorylation reaction. Clearly, more experimental work is needed to understand the antilipolytic, insulin-like effect of TPA.

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