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DIFFERENTIAL MODULATION OF THE ADENYLATE CYCLASE/CYCLIC AMP STIMULATORY PATHWAY BY PROTEIN KINASE C ACTIVATION IN RAT ADIPOSE TISSUE AND ISOLATED FAT CELLS

INFLUENCE OF COLLAGENASE DIGESTION

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Abstract—Exposure of rat epididymal fat pad to phorbol 12-myristate 13-acetate (TPA), an activator of protein kinase C, results in an 85% increase in isoproterenol-stimulated cyclic AMP (cAMP) accumulation, an effect which was antagonized by H7, a protein kinase C inhibitor. This promoting action of TPA appears to be related to (i) an increase in the catalytic activity of adenylate cyclase, (ii) an increase in the maximal response of adenylate cyclase to fluoride and guanylimidodiphosphate (GppNHp) with no change in the EC_{50} value for GppNHp, and (iii) a reduction of the isoproterenol-stimulated low-K_m cAMP phosphodiesterase activity present in the 30,000 *g* pellet of fat pad homogenates. In contrast with fat pads, exposure of isolated rat fat cells to TPA failed to influence their adenylate cyclase response to GppNHp and their cAMP accumulation and lipolysis. However, the other alterations caused by TPA in fat pads were still observed in fat cells. These results suggest that (i) the major alteration responsible for the promoted isoproterenol-stimulated cAMP response observed in fat pads after exposure to TPA is an increased interaction between the αs subunit of Gs and the catalytic site of adenylate cyclase and (ii) this increased interaction is dependent on protein kinase C activation and is abolished by collagenase digestion.

There is now growing evidence for interactions between the cyclic AMP (cAMP) and diacylglycerol-mediated hormonal responses in various tissues and cell lines. The mechanisms underlying this "cross talk" appear different, however, according to the tissues. For example, activation of protein kinase C by the phorbol ester phorbol 12-myristate 13-acetate (TPA) produces down regulation of β-adrenoceptors in some (for a review see Ref. 1) but not all tissues [2, 3]. Receptors such as the gonadotropin receptor in a Leydig tumor cell line [4] and the vasoactive intestinal peptide receptor in GH3 pituitary tumor cells [5] are also desensitized after exposure to phorbol esters. Activation of protein kinase C by phorbol esters has also been shown to induce the inactivation of Gi in hepatocytes [6], S49 lymphoma cells [7], GH4Cl cells [8], brain striatal membranes [9], keratinocytes [10] and platelets [11, 12]. Last, exposure to TPA or activation of protein kinase C results in an increased interaction between Gs and the adenylate cyclase catalytic subunit (C) in S49 lymphoma cells [13], a phosphorylation of C in GH3 cells [14], frog erythrocytes and bovine brain [15] and an activation of phosphodiesterase in C6 glioma cells [16].

In adipocytes, where translocation and activation of protein kinase C are promoted by TPA [17], glucose [18] and several hormones [19–23], the situation regarding a possible interaction between the cAMP- and diacylglycerol-mediated hormonal responses appears rather controversial. As a matter of fact, three reports have provided some evidence suggesting the existence of such a "cross talk" in adipose cells. First, exposure of intact adipose tissue to TPA has been shown to induce the activation of low-K_m cAMP phosphodiesterase activity [24]. Second, in isolated fat cells, activation of adenylate cyclase by purified pancreatic protein kinase C has been reported [25]. Last, isoproterenol-stimulated lipolysis is inhibited by inhibitors of protein kinase C in fat pads [26]. However, four other recent studies, all performed on isolated rat fat cells, were unable to reveal any significant influence of TPA on basal and stimulated cAMP accumulation or lipolysis [27–30].

In order to determine whether these discrepancies could be due to differences in the experimental designs used in the above-mentioned studies, we have presently re-examined the possibility of a "cross talk" between the cAMP and protein kinase C pathways by comparing the influence of TPA on the enzymatic steps controlling cAMP in both isolated adipocytes and adipose tissue fragments.

MATERIALS AND METHODS

Materials. Phorbol 12-myristate 13-acetate (TPA), 1 (5-isoquinolinylsulfonyl) 2-methyl piperazine (H7), benzamidine, trypsin inhibitor, bovine serum albumin, isobutylmethylxanthine (IBMX), phosphocreatine, creatine phosphokinase, isoproterenol bitartrate, EGTA, TES, snake venom (*Crotalus*

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Table 1. Effects of TPA preincubation (1 μM, 30 min) in the absence or presence of H7 (0.1 μM) on isoproterenol (1 μM)-induced cAMP accumulation in fat pads

Preincubation with	0	TPA (1 μM)	TPA (1 μM) + H7 (0.1 μM)
Incubation	cAMP accumulation		
Isoproterenol (1 μM)	100	185 ± 11*	116 ± 8†
Isoproterenol (1 μM) + ASA	193 ± 31*	354 ± 58*	ND

Fat pads were incubated with 50 mI.U./mL adenosine deaminase (ADA) and 1 μM isoproterenol and, when indicated 0.3 mM acetylsalicylate (ASA). Each bar is expressed as the percentage of controls (preincubation without TPA, incubation with isoproterenol) and represents the mean values ± SEM of four different experiments performed in triplicate. cAMP accumulation in controls was 367 ± 39 pmol/g/min.

* $P < 0.01$ compared to controls; † not significant compared to controls.

ND, not determined.

atrox), dimethyl sulphoxide (DMSO) and ATP were from Sigma. Adenosine deaminase (ADA), GTP, GppNHp, GDPβS, N^6-phenylisopropyl-adenosine (R-PIA) and cAMP were from Boehringer Mannheim. Collagenase was from Worthington, and the [^{3}H]cAMP and cAMP assay system were from Amersham. AG1X2 was from Biorad and forskolin from Calbiochem. All other products were of analytical grade.

R-PIA, IBMX and TPA were dissolved in DMSO (1% final), forskolin in ethanol (4/1000 final), and isoproterenol in ascorbic acid (1/10,000 final).

Preincubations. Epididymal adipose tissue was removed from male Wistar rats (180–225 g). Fat cells were isolated by collagenase digestion and adipocyte membranes prepared by homogenization (pads) or mechanical disruption (fat cells) as described previously [31]. Pads (1 g tissue/mL) or adipocytes ($0.1–0.4 \times 10^6$ cells/mL) were preincubated at 37° in KRT–HCl (pH 7.4), containing 4% albumin and 50 mI.U./mL ADA (unless otherwise specified) for 30 min in the presence of 1 μM TPA. TPA was diluted in DMSO to obtain 1% DMSO, and DMSO alone was used as the control. The preincubation and incubation media were glucose-free to avoid protein kinase C activation and translocation [18]. The TPA concentrations mentioned refer to the concentrations added and do not take into account the amount of TPA bound to albumin.

In the phosphodiesterase studies, the preincubation was prolonged for 10 min in the presence or absence of 1 μM isoproterenol.

Lipolysis, cAMP production and adenylate cyclase. After the 30 min preincubation period, fat pads (20–50 mg) or isolated fat cells ($0.1–0.4 \times 10^6$ cells) were further incubated at 37° for 1 hr (lipolysis) or 10 min (cAMP) in the presence of the indicated effectors. Then, glycerol release and cAMP production were measured as described previously [31]. Under all conditions tested, less than 10% of the total cAMP (intracellular and in the medium) accounted for extracellular cAMP.

Adenylate cyclase assays were conducted as follows: membranes (12–25 μg protein) were incubated at 37° in a final volume of 100 μL containing 25 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$ or 2 mM Mn^{2+}, 1 mM EGTA, 1 mM IBMX, 100 m.I.U./mL ADA, 0.3 mM ATP, 5 mM creatine phosphate, 5 I.U./mL creatine phosphokinase and 1 mg/mL albumin. After 10 min reactions were stopped and cAMP determined as described above.

Low-K_m cAMP phosphodiesterase assays. Preincubated pads and adipocytes were homogenized in ice-cold 10 mM TES, 250 mM sucrose (pH 7.5 at 30°), centrifuged (2 min, 400 *g*, 4°) and the resulting infranatants used for further fractionation. The following fractions were prepared: a 30,000 *g* pellet and supernatant, a 30,000 *g* supernatant obtained from sonicated homogenates according to Solomon and Palazzolo [24], the P2 and P3 fractions prepared according to Kono [32] and Anderson *et al.* [33], respectively, and a 100,000 *g* pellet and supernatant. Before being assayed, all these fractions were diluted in ice-cold 10 mM TES, 250 mM sucrose (pH 7.5 at 30°) to yield a final protein concentration of 0.3–0.6 mg/mL.

Phosphodiesterase activity was assayed according to a slight modification of the two-step reaction procedure of Thompson and Appleman [34]. Briefly, 25 μL of the phosphodiesterase-containing fraction were incubated at 30° in a final volume of 100 μL containing 5 mM $MgSO_4$, 0.08–0.50 μM cAMP, 35,000–40,000 cpm [^{3}H]cAMP and 50 mM TES (pH 7.5). After 10 min, reactions were stopped and enzyme activity determined as described previously [31]. All fractions displayed linear Lineweaver–Burk plots for cAMP concentrations ranging from 0.08 to 0.50 μM with K_m values in the 0.4–0.6 μM range (not shown).

Other determinations and statistics. Proteins and cell numbers were determined as described previously [31]. All results are presented as mean ± SEM. All experiments were performed in triplicate and repeated twice at least. Comparison between control and TPA-preincubated values were performed using unpaired Student's *t*-test.

RESULTS

Studies on whole adipose tissue

cAMP accumulation. As shown in Table 1, a 30 min preincubation in the presence of TPA

Table 2. Isoproterenol-stimulated glycerol release from fat pads after 30 min preincubation in the absence (control) or presence of 1 μM TPA

Preincubation	Control	TPA
Incubation	Glycerol release (μmol/g/60 min)	
Basal	1.82 ± 0.23	1.64 ± 0.32 (90 ± 8%, NS)
Isoproterenol (1 μM)	7.52 ± 0.15	7.70 ± 0.20 (103 ± 6%, NS)
Isoproterenol (1 μM) + R-PIA (10 nM)	5.91 ± 0.33	7.47 ± 0.33 (126 ± 3%, $P < 0.05$)

Results are mean values ± SEM of three different experiments performed in triplicate. Numbers in parentheses are the percentages of control values. In these three experiments, cAMP accumulation in the presence of R-PIA were 47 ± 12% and 59 ± 8% of the cAMP found without R-PIA in control and TPA-exposed fat pads, respectively.
NS, not significant.

Table 3. Influence of TPA preincubation on the 30,000 *g* pellet low-K_m cAMP phosphodiesterase activity in fat pads

Preincubation	Isoproterenol	TPA	TPA + isoproterenol
% over basal	+28 ± 4% $0.005 < P < 0.01$*	+2 ± 9% NS*	+6 ± 7% (61 ± 12%) NS* $P < 0.001$†

Fat pads were preincubated in the absence (control) or presence of 1 μM TPA. After 30 min, the incubations were prolonged for 10 min in the absence (basal) or presence of 1 μM isoproterenol and the low-K_m cAMP phosphodiesterase measured in the 30,000 *g* pellet. Each value represents the isoproterenol activation expressed as percentage increase over the basal values found in controls and are mean values of seven experiments performed in triplicate. Mean basal activity in controls was 26.9 ± 2.8 pmol/mg/min. Numbers in parentheses represent the percentage inhibition due to TPA of the isoproterenol activatory effect.
* Compared to basal value in controls.
† Compared to isoproterenol value in controls.
NS, not significant.

followed by a 10 min incubation with 1 μM isoproterenol resulted in an 85 ± 11% (N = 4) increase in cAMP accumulation in the fat pads. Addition of 0.3 mM acetylsalicylate in the preincubation promoted *per se* an increase in basal and isoproterenol-stimulated cAMP accumulation but failed to alter the promoting effect of TPA. When ADA was replaced by 200 mM adenosine, similar potentiation of the isoproterenol response by TPA was observed (not shown). In contrast, preincubation with TPA did not modify the basal or IBMX (1 mM)-stimulated cAMP accumulation. Last, when 0.1 μM H7 was added with TPA, the promoting effect of TPA on isoproterenol-stimulated cAMP accumulation was almost completely antagonized (Table 1). It must be noted that preincubation with H7 alone had no influence on the isoproterenol-induced cAMP response of fat pads (data not shown).

As shown in Table 2, the lipolytic response of fat pads to isoproterenol was unmodified by a 30 min TPA preincubation. Varying the preincubation from 0 to 60 min failed to reveal any effect of TPA on the basal, 1 mM IBMX-stimulated or 1 μM isoproterenol-stimulated lipolysis (not shown).

This lack of regulatory effect of TPA on lipolysis seems to be explained by the finding that the promoting action of TPA on cAMP is seen for cAMP concentrations far over those inducing maximal lipolysis [31, 35]. In fact, when cAMP was lowered by the addition of 10 nM R-PIA, the lipolytic activity in response to isoproterenol was slightly but significantly enhanced by TPA (Table 2). These results which cannot be attributed to a decreased inhibitory effect of R-PIA on cAMP accumulation (see legend to Table 2), reveal the existence of a cross-regulation between TPA and the β-adrenergic stimulated cAMP-dependent lipolytic pathway in fat pads.

As shown in Table 3, in the 30,000 *g* pellet of unsonicated fat pad homogenates, a 10 min preincubation in the presence of isoproterenol alone induced a slight but significant increase in the low-K_m cAMP phosphodiesterase activity. In contrast, a 40 min preincubation in the presence of TPA alone had no effect on this activity. However, when both TPA and isoproterenol were present in the preincubation, the stimulation due to isoproterenol alone was partly suppressed. In addition, the low-K_m cAMP phosphodiesterase activities in the 30,000 *g*

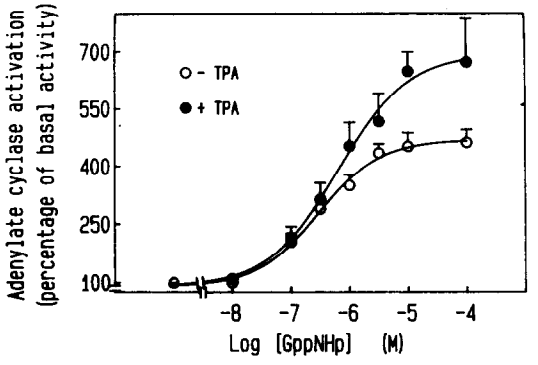

Fig. 1. Adenylate cyclase response to GppNHp in membranes prepared from TPA- or vehicle-preincubated fat pads. Results are expressed as percentages of basal activity. This figure represents the mean values ± SEM of five different experiments performed in triplicate. Basal adenylate cyclase activities were 82, 69, 38, 61 and 88 pmol AMP/mg/min in controls and 78, 65, 46, 50 and 22 pmol cAMP/mg/min in TPA (1 μM, 30 min) preincubated fat pads. EC_{50} values for GppNHp were 0.156 ± 0.063 and 0.178 ± 0.071 μM in controls and TPA preincubated fat pads, respectively.

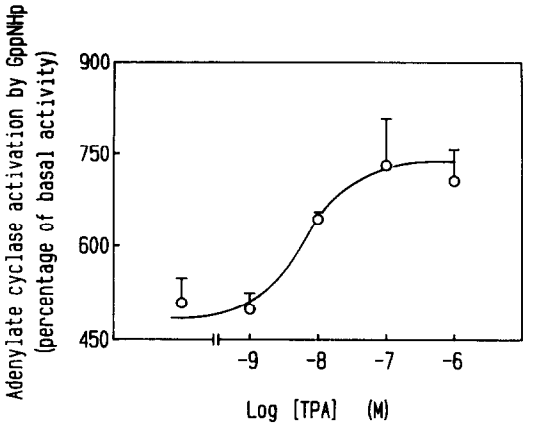

Fig. 2. Dose-dependent promoting effect of TPA preincubation (30 min) on the adenylate cyclase response to 1 μM GppNHp in fat pad membranes. Experimental conditions are described in Materials and Methods. Results are mean values ± SEM of four different experiments performed in triplicate. Calculated EC_{50} value for TPA = 8 ± 2 nM.

supernatant, the P2 and P3 fractions and the 100,000 g pellet and supernatant were found unaltered by a preincubation with TPA. However, when homogenates were sonicated (30 sec) prior to being centrifuged, pre-exposure to TPA resulted in a 47 ± 16% increase in the low-K_m cAMP phosphodiesterase activity of the 30,000 g supernatant.

Comparison of adenylate cyclase activities in membranes prepared from undigested fat pads preincubated for 30 min with or without 1 μM TPA revealed a promoting effect of TPA:

(i) on the catalytic activity (+37 ± 4%, N = 4) as shown by the adenylate cyclase response to 2 mM Mn^{2+}, 100 μM forskolin + 500 μM GDPβS [36];

(ii) on the adenylate cyclase maximal response to 10 μM GppNHp (+49 ± 15%, N = 7) with no apparent change in the sensitivity to GppNHp (Fig. 1), an effect which is dose-dependent (Fig. 2);

(iii) on the adenylate cyclase maximal response to 10 mM NaF (+28 ± 3%, N = 3).

However, no significant effect of TPA could be observed on the adenylate cyclase response to isoproterenol as revealed by dose–response curve studies performed in the presence of either 10 mM Mg^{2+} (isoproterenol EC_{50} values 0.41 ± 0.21 μM in control vs 0.34 ± 0.05 μM in TPA-preincubated pads, N = 4, not significant or 2 mM Mg^{2+} (data not shown).

Studies on isolated fat cells

Contrasting with the above results, preincubation with TPA failed to affect basal and isoproterenol-stimulated cAMP and lipolysis in isolated fat cells, whatever the experimental conditions used (preincubation times varying from 0 to 60 min, with

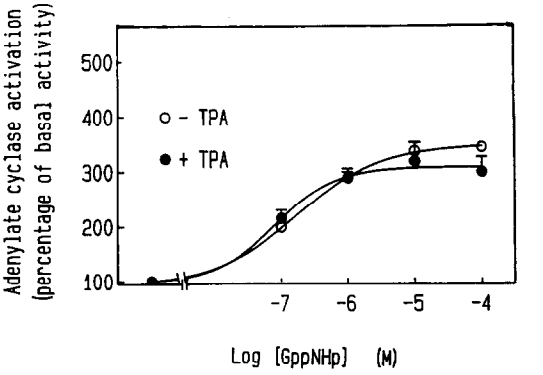

Fig. 3. Influence of TPA preincubation (1 μM, 30 min) on the adenylate cyclase response to GppNHp in membranes prepared from isolated adipocytes. Results are expressed as percentages of basal activity. This figure represents mean values ± SEM of three different experiments performed in triplicate. Basal adenylate cyclase activities were: 67, 32 and 81 pmol cAMP/mg/min in controls and 48, 51 and 90 pmol cAMP/mg/min in TPA-preincubated fat cells.

or without H7, in the presence of R-PIA or glucose). These negative findings pointed to a possible interference of the collagenase treatment with the mechanism whereby TPA affected the isoproterenol-stimulated cAMP pathway in fat pads. To test this hypothesis, we conducted parallel experiments in which isolated fat cells were compared to fat pads. The following results were obtained:

(i) the increased adenylate cyclase response to GppNHp observed in fat pad membranes after TPA preincubation completely disappeared after collagenase digestion (Fig. 3);

(ii) the increased adenylate cyclase catalytic

activity (2 mM Mn^{2+} + 100 μM forskolin + 500 μM GDPβS) seen in fat pads after TPA preincubation still persisted in isolated fat cells (not shown);

(iii) in the absence of any exposure to TPA, collagenase digestion reduced by half the low-K_m cAMP phosphodiesterase activity of the 30,000 g pellet (16.5 ± 3.2 pmol/mg/min in adipocytes vs 30.3 ± 3.7 pmol/mg/min in pads, N = 4, $P < 0.05$), a result which is in accordance with another report [37]. However, the collagenase treatment resulted in a potentiation of the isoproterenol stimulatory effect which was increased from +28 ± 4% in pads to +63 ± 5% ($P < 0.001$) in isolated adipocytes;

(iv) after collagenase treatment, the TPA-induced partial reversal of the isoproterenol stimulatory effect on the low-K_m cAMP phosphodiesterase activity still persisted but was weaker than in fat pads (35 ± 13% of inhibition, in fat cells vs 61 ± 16% in fat pads).

Finally, since an intercell cooperation for metabolic regulation has been described in adipose cells [38], we could not exclude that the promoting effect of TPA on isoproterenol-stimulated cAMP seen in fat pads but not in fat cells might be attributed to the non-adipose connective cells. However, this hypothesis could be ruled out because (i) under all our incubation conditions, cAMP synthesized by the non-adipose cells was undetectable, and (ii) coincubation of isolated adipocytes and isolated non-adipose cells (mixed in the same proportion than in intact adipose tissue) failed to reveal any effect of TPA on both isoproterenol-stimulated cAMP accumulation and lipolysis (data not shown).

DISCUSSION

Our results show a cross regulation between TPA-activated pathways and isoproterenol-induced cAMP accumulation and glycerol release in undigested fat tissue. As a matter of fact, we observed an increase in pads preincubated with TPA. Moreover, in membranes prepared from those pads, the adenylate cyclase stimulatory responses to either GppNHp or Mn^{2+} + GDPβS + forskolin were found increased, whereas the stimulatory effect of isoproterenol on the low-K_m cAMP phosphodiesterase activity was partly suppressed. Finally, among these TPA effects, only the last two could be reproduced in isolated fat cells, suggesting that collagenase digestion interferes with some but not all the targets of TPA in adipose tissue.

From the present data, it seems that the mechanisms whereby TPA cross regulates the isoproterenol-stimulated cAMP pathway in fat pads are: (i) an increase in adenylate cyclase catalytic activity, (ii) an increase in the interaction between Gs, the stimulatory regulatory protein and C, the catalytic subunit of adenylate cyclase and (iii) a decrease in phosphodiesterase activation by isoproterenol. In addition, it can be concluded that these mechanisms are linked to protein kinase C activation, since, first, the metabolic effects of TPA are generally assumed to result from protein kinase C activation [39], and second, H7, a potent inhibitor of protein kinase C [40], antagonizes the TPA effect on isoproterenol-stimulated cAMP response.

The substrates for phosphorylation by protein kinase C are multiple: in erythrocytes and brain, phosphorylation of the adenylate cyclase catalytic subunit by protein kinase C has been shown to result in an increase in catalytic activity [15], a mechanism which could also occur in adipose tissue and adipocytes as suggested by the present study and a previous report [25]. In some cells, β-adrenoceptors are also phosphorylated by protein kinase C [41] and this phosphorylation appears to contribute to receptor desensitization (for a review, see Ref. 1). Such a mechanism, however, does not seem to occur in adipose tissue *in vitro*, since under our incubation conditions (10 mM Mg^{2+}, or 2 mM Mg^{2+} to reveal uncoupling) adenylate cyclase dose–response curves to isoproterenol were unaltered by TPA.

Another protein which is also phosphorylated by protein kinase C is Gi [11, 42]. However, if this also occurs in adipose tissue, such a phosphorylation does not seem to play a significant role in the mechanism whereby TPA enhances adenylate cyclase catalysis since, under the incubation conditions used (high Mg^{2+} and GTP concentrations, no Na^+) Gi does not interact with adenylate cyclase. To our knowledge, there is no report suggesting Gs phosphorylation by protein kinase C. However, adenylate cyclase phosphorylation could be sufficient to induce both the increase in catalytic activity and the potentiation of the interaction between αs and C.

In hepatocytes, TPA was reported to inhibit the low-K_m cAMP phosphodiesterase activity [43]. This contrasts with the present study showing that this activity is selectively increased in the 30,000 g supernatant of sonicated fat pads after exposure to TPA.

Activation of the low-K_m cAMP phosphodiesterase by isoproterenol is well established in adipose tissue [32, 33, 44, 45]. This activation which appears to be catalysed by protein kinase A, [46, 47] is, as shown in the present report, suppressed by TPA. This leads to the suggestion that this enzyme, or the system involved in its activation by isoproterenol, could be a substrate for protein kinase C, but there is so far no report supporting this suggestion.

In contrast with the above results, the cAMP content and lipolytic activity of fat pads were found to be insensitive to TPA when studied under unstimulated conditions. This can be explained, however, by the balance between the two opposite effects of TPA on cAMP metabolism observed in this study: the activation of the catalytic activity of adenylate cyclase on one hand and the activation of the low-K_m cAMP phosphodiesterase on the other one.

In the isolated adipocytes, TPA preincubation still increases adenylate cyclase catalytic activity and also reduces the magnitude of the isoproterenol stimulation of the low-K_m cAMP phosphodiesterase. However, no effect of TPA on cAMP accumulation or glycerol release was seen, a result which confirms previous studies failing to observe any change in isolated fat cell lipolytic activity after exposure to TPA [27–30]. In addition, this paradoxical observation suggests that collagenase-treatment interferes with the lipolytic cascade by altering the interaction between the αs and the adenylate cyclase

catalytic subunits, i.e. between the GDP/GTP exchange reaction which is fast at 37° [48], and the GTP hydrolytic step (since our experiments are performed with the non-hydrolysable GTP analog GppNHp). One possible explanation is that collagenase (or a contaminating activity [37] present in the three different collagenase lots presently used) may have altered a membranous factor and that this alteration may no longer allow TPA to promote the Gs–C interaction. More than Gs itself, this factor could be an anchoring factor for Gs, or an inhibitor of the Gs–protein kinase C interaction which could be released during the collagenase treatment.

Finally, since isolated adipocytes do not present any modification of their cAMP content and glycerol release after exposure to TPA, our results suggest (i) that the amplified αs–C interaction seen in TPA-treated fat pads is crucial in the promoting effect of TPA on isoproterenol-stimulated cAMP pathway and (ii) that collagenase digestion is unsuitable for studies on the cross-regulation between adenylate cyclase stimulatory receptor-mediated pathways and the protein kinase C system in adipose tissue.

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REFERENCES

1. Sibley DR, Benovic JL, Caron MG and Lefkowitz RJ, Regulation of transmembrane signaling by receptor phosphorylation. *Cell* **48**: 913–919, 1987.
2. Garcia-Sainz JA, Alcantara R, Hernandez-Sotomayor SMT and Mas-Oliva J, Beta-1-adrenoceptors in rat hepatoma. Desensitization by isoproterenol and phorbol-myristate-acetate. *Life Sci* **44**: 1767–1775, 1989.
3. Kassis S, Zaremba T, Patel J and Fishman PH, Phorbol esters and β-adrenergic agonists mediate desensitization of adenylate cyclase in rat glioma C6 cells by distinct mechanisms. *J Biol Chem* **260**: 8911–8917, 1985.
4. Rebois VR and Patel J, Phorbol esters cause desensitization of gonadotrophin-responsive adenylate cyclase in a murine Leydig tumor cell line. *J Biol Chem* **260**: 8026–8031, 1985.
5. Quilliam LA, Dobson PRM and Brown BL, Modulation of cyclic AMP accumulation in GH3 cells by a phorbol ester and thyreoliberin. *Biochem Biophys Res Commun* **129**: 898–903, 1985.
6. Pyne NJ, Murphy GJ, Milligan G and Houslay MD, Treatment of intact hepatocytes with either the phorbol ester TPA or glucagon elicits the phosphorylation and functional inactivation of the inhibitory guanine nucleotide regulatory protein Gi. *FEBS Lett* **243**: 77–82, 1989.
7. Bell JD and Brunton LL, Enhancement of adenylate cyclase activity in S49 lymphoma cells by phorbol esters. Withdrawal of GTP-dependent inhibition. *J Biol Chem* **261**: 12036–12041, 1986.
8. Gordeladze JO, Bjôro T, Torjesen PA, Ostberg BC, Haug E and Gautvik KM, Protein kinase C stimulates adenylate cyclase activity in prolactin-secreting rat adenoma (GH4Cl) pituicytes by inactivating the inhibitory GTP-binding protein Gi. *Eur J Biochem* **183**: 397–406, 1989.
9. Olianas MC and Onali P, Phorbol esters increase GTP-dependent adenylate cyclase activity in rat brain striatal membranes. *J Neurochem* **47**: 890–897, 1986.
10. Choi EJ and Toscano WA, Modulation of adenylate cyclase in human keratinocytes by protein kinase C. *J Biol Chem* **263**: 17167–17172, 1988.
11. Katada T, Gilman AG, Watanabe Y, Bauer S and Jakobs KH, Protein kinase C phosphorylates the inhibitory guanine-nucleotide-binding regulatory component and apparently suppresses its function in hormonal inhibition of adenylate cyclase. *Eur J Biochem* **151**: 431–437, 1985.
12. Jakobs KH, Bauer S and Watanabe Y, Modulation of adenylate cyclase of human platelets by phorbol ester. Impairment of the hormone-sensitive pathway. *Eur J Biochem* **151**: 425–430, 1985.
13. Bell JD, Buxton ILO and Brunton LL, Enhancement of adenylate cyclase activity in S49 lymphoma cells by phorbol esters. *J Biol Chem* **260**: 2625–2628, 1985.
14. Quilliam LA, Dobson PRM and Brown BL, Regulation of GH3 pituitary tumour-cell adenylate cyclase activity by activators of protein kinase C. *Biochem J* **262**: 829–834, 1989.
15. Yoshimase T, Sibley DR, Bouvier M, Lefkowitz RJ and Caron MG, Cross-talk between cellular signalling pathways suggested by phorbol-ester-induced adenylate cyclase phosphorylation. *Nature* **327**: 67–69, 1987.
16. Bressler JP and Tinsely P, Regulation of cAMP levels by protein kinase C in C6 rat glioma cells. *J Neurosci Res* **25**: 81–86, 1990.
17. Skoglung G, Hansson A and Ingelman-Sundberg M, Rapid effects of phorbol esters on isolated rat adipocytes. Relationship to the action of protein kinase C. *Eur J Biochem* **148**: 407–412, 1985.
18. Ishizura T, Hoffman J, Cooper DR, Watson JE, Pushkin DB and Farese RV, Glucose induced synthesis of diacylglycerol *de novo* is associated with translocation (activation) of protein kinase C in rat adipocytes. *FEBS Lett* **249**: 234–238, 1989.
19. Pershadsingh HA, Shade DL and McDonald JM, Insulin-dependent alterations of phorbol ester binding to adipocyte subcellular constituents. Evidence for the involvement of protein kinase C in insulin action. *Biochem Biophys Res Commun* **145**: 1384–1389, 1987.
20. Draznin B, Leitner JW, Sussman KE and Sherman NA, Insulin and glucose modulate protein kinase C activity in rat adipocytes. *Biochem Biophys Res Commun* **156**: 570–575, 1988.
21. Egan JJ, Saltis J, Wek SA, Simpson IA and Londos C, Insulin, oxytocin and vasopressin stimulate protein kinase C activity in adipocyte plasma membranes. *Proc Natl Acad Sci USA* **87**: 1052–1056, 1990.
22. Augert G and Exton JH, Insulin and oxytocin effects on phosphoinositide metabolism in adipocytes. *J Biol Chem* **263**: 3600–3609, 1988.
23. Lee HM and Fain JN, Regulation of oxytocin-induced phosphoinositide breakdown in adipocytes by adenosine, isoproterenol and insulin. *Biochim Biophys Acta* **1013**: 73–79, 1989.
24. Solomon SS and Palazzolo M, Activation of cyclic AMP phosphodiesterase by phorbol and protein kinase C pathway. *Am J Med Sci* **292**: 182–184, 1986.
25. Naghshineh S, Noguchi M, Huang KP and Londos C, Activation of adipocyte adenylate cyclase by protein kinase C. *J Biol Chem* **261**: 14534–14538, 1989.
26. Gorin E, Tai LR, Honeyman TW and Goodman HM, Evidence for a role of protein kinase C in the stimulation of lipolysis by growth hormone and isoproterenol. *Endocrinology* **126**: 2973–2982, 1990.
27. Garcia-Sainz JA and Juarez-Ayala J, Effects of phorbol esters on the hormonal responsiveness of isolated white fat cells. *Eur J Pharmacol* **146**: 193–199, 1988.
28. Kelly KL, Stimulation of adipocyte phospholipid

methyltransferase activity by phorbol 12-myristate 13-acetate. Differential regulation of phospholipid methyltransferase and lipolysis. *Biochem J* **241**: 917–921, 1987.
29. Hall M, Taylor SJ and Saggerson ED, Persistent activity modification of phosphatidate phosphohydrolase and fatty acyl-CoA synthetase on incubation of adipocytes with the tumour promoter 12-0 tetradecanoylphorbol 13-acetate. *FEBS Lett* **179**: 351–354, 1985.
30. Carpene C, Lambert B and Lafontan M, The phorbol diester PMA does not alter the adrenergic regulation of lipolysis in hamster adipocytes. *Fundam Clin Pharmacol*, **3**: 549–550, 1989.
31. De Mazancourt P, Giot J and Giudicelli Y, Role of the cyclic AMP and Ri-receptor-Gi-coupled adenylate cyclase inhibitory pathway in the mechanism whereby adrenalectomy increases the adenosine antilipolytic effect in rat fat cells. *Endocrinology* **124**: 1131–1139, 1989.
32. Kono T, Insulin-sensitive cAMP phosphodiesterase in rat adipose tissue. *Methods Enzymol* **159**: 745–751, 1988.
33. Anderson NG, Kilgour E and Houslay MD, Subcellular localization and hormone sensitivity of adipocyte cyclic AMP phosphodiesterase. *Biochem J* **262**: 867–872, 1989.
34. Thompson WJ and Appleman MM, Multiple cyclic nucleotide phosphodiesterase activities from rat brain. *Biochemistry* **10**: 311–316, 1971.
35. Honnor RC, Dhillon GS and Londos C, cAMP-dependent protein kinase and lipolysis in rat adipocytes: 1. Cell preparation, manipulation and predictability in behavior. *J Biol Chem* **260**: 15122–15129, 1985.
36. Chang FH and Bourne HR, Dexamethasone increases adenylyl cyclase activity and expression of the α-subunit of Gs in GH3 cells. *Endocrinology* **121**: 1711–1715, 1987.
37. Engfeldt P, Arner P and Östman J, Influence of adipocyte isolation by collagenase on phosphodiesterase activity and lipolysis in man. *J Lipid Res* **21**: 443–448, 1980.
38. Parker J, Lane J and Axelrod L, Cooperation of adipocytes and endothelial cells required for catecholamine stimulation of PGI2 production by rat adipose tissue. *Diabetes* **38**: 1123–1132, 1989.
39. Castagna M, Takai Y, Kaibuchi K, Sano K, Kikkawa U and Nishizuka Y, Direct activation of calcium-stimulated phospholipid-dependent protein kinase by tumor promoting phorbol esters. *J Biol Chem* **257**: 7847–7851, 1982.
40. Hidaka M, Inagaki M and Sasaki Y, Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. *Biochemistry* **23**: 5036–5041, 1984.
41. Bouvier M, Leeb-Lundberg LMF, Benovic JL, Caron MG and Lefkowitz RJ, Regulation of adrenergic receptor function by phosphorylation: II. Effects of agonist occupancy on phosphorylation of α-1 and β-2-adrenergic receptors by protein kinase C and the cyclic AMP-dependent protein kinase. *J Biol Chem* **262**: 3106–3113, 1987.
42. Rothenberg PL and Kahn CR, Insulin inhibits pertussis toxin-catalyzed ADP-ribosylation of G-proteins. *J Biol Chem* **263**: 15546–15552, 1988.
43. Irvine F, Pyne NJ and Houslay MD, The phorbol ester TPA inhibits cyclic AMP phosphodiesterase activity in intact hepatocytes. *FEBS Lett* **208**: 255–259, 1986.
44. Boyes S and Loten EG, Insulin and lipolytic hormones stimulate the same phosphodiesterase isoform in rat adipose tissue. *Biochem Biophys Res Commun* **162**: 814–820, 1989.
45. Manganiello V, Degerman E and Elks M, Selective inhibitors of specific phosphodiesterases in intact adipocytes. *Methods Enzymol* **159**: 504–520, 1988.
46. Gettys TW, Vine AJ, Simonds MKF and Corbin JD, Activation of the particulate low-K_m phosphodiesterase of adipocytes by addition of cAMP dependent protein kinase. *J Biol Chem* **263**: 10359–10363, 1988.
47. Degerman E, Smith CJ, Tornqvist H, Vasta V, Belfrage P and Manganiello VC, Evidence that insulin and isoprenaline activate the cGMP-inhibited low-K_m cAMP phosphodiesterase in rat fat cells by phosphorylation. *Proc Natl Acad Sci USA* **87**: 533–537, 1990.
48. Hudson TH and Fain JN, Forskolin-activated adenylate cyclase. Inhibition by guanyl-5-yl imidodiphosphate. *J Biol Chem* **258**: 9755–9761, 1983.