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CYTOKINES STIMULATE LIPOLYSIS AND DECREASE LIPOPROTEIN LIPASE ACTIVITY IN CULTURED FAT CELLS BY A PROSTAGLANDIN INDEPENDENT MECHANISM

Ingibjörg Hardardóttir, William Doerrler, Kenneth R. Feingold and Carl Grünfeld

Department of Medicine, University of California and Metabolism Section, Medical Service, V. A. Medical Center, San Francisco, California 94121

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We previously showed that indomethacin blocked the effect of tumor necrosis factor (TNF) and other cytokines on lipolysis. We now show that TNF stimulates prostaglandin (PG) production, enhances lipolysis and decreases lipoprotein lipase (LPL) activity in 3T3-F442A adipocytes and indomethacin blocks these activities, suggesting that the actions of TNF are mediated by PG's. However, exogenous PGE_2 at the levels induced by TNF is not sufficient to affect lipolysis or LPL activity and low doses of indomethacin and flurbiprofen block PG production without affecting TNF's action. Interleukin-1 and interferon-α and γ induce lipolysis and decrease LPL activity but do not stimulate much PG production. These results demonstrate that cytokines enhance lipolysis and decrease LPL activity in 3T3 adipocytes by a PG independent mechanism.

Previous studies have shown that tumor necrosis factor (TNF) stimulates lipolysis (1-3) and suppresses lipoprotein lipase (LPL) activity (1,2,4,5), mRNA levels (4) and enzyme synthesis (5-7) in murine 3T3 adipocytes. Interleukin-1 (IL-1) stimulates lipolysis (8) and suppresses LPL activity (8,9) in these cells by decreasing the synthesis of the LPL enzyme (8) without decreasing mRNA levels (4). Interferon-α (IFN), -β and -γ stimulate lipolysis (1,3) and IFN-α and -γ decrease LPL activity in 3T3 adipocytes (1).

Prostaglandin (PG) E_2 is a potent inhibitor of basal and hormone-induced lipolysis in rat and human adipocytes (10-12) but PGE_1 enhances lipolysis in 3T3-L1 adipocytes (13). PGs are released from adipocytes under hormonal and neuronal stimulation (14,15). In the preadipocyte stage of the clonal line OB17, PGE_2 is the major PG produced and 6-keto $PGF_{1\alpha}$ is only found at low levels (16). Lower levels of PG synthesis occur, however, after adipose conversion. There are no reports of cytokine stimulation of PG synthesis in 3T3 adipocytes. However, TNF and IL-1 stimulate PGE_2 synthesis in the parent line, Swiss 3T3 fibroblasts (17,18).

Abbreviations used in this paper: IFN, interferon; IL, interleukin; LPL, lipoprotein lipase; PG, prostaglandin; TNF, tumor necrosis factor.

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Many in vitro effects of cytokines may be mediated by activation of the cyclooxygenase pathway (19-21). TNF and/or IL-1 stimulate PGE_2 production in human synovial cells, dermal fibroblasts and vascular smooth muscle cells (22-24) and prostacyclin synthesis in cultured vascular endothelial cells (19). Bone resorption in mouse calvaria is stimulated by TNF via a PG mediated mechanism (21). Some effects of TNF and IL-1 in vivo are also mediated by PGs. The classic febrile response is induced by IL-1 and TNF by increasing the synthesis of PGE_2 in the hypothalamic vasculature, and blocked by inhibitors of PG synthesis (20,25) and the shock syndrome in rodents, induced by TNF or IL-1, can be prevented by pretreatment with PG synthesis inhibitors (26,27). Signal transduction by IFN-α may also be mediated through arachidonic acid metabolism (28).

A previous study (3) showed that stimulation of lipolysis by TNF, IL-1 and the IFNs was blocked by indomethacin, an inhibitor of PG synthesis. The present investigation explores the relationship between cytokine action and PGs in 3T3 adipocytes.

Materials and Methods

Cytokines and other materials. Murine TNF-α (SA, 2.9×10^7 U/mg) and murine IFN-γ (SA, 5×10^6 U/mg) were kindly provided by Genentech, Inc. (South San Francisco, CA). Recombinant human IL-1β (112-269) (SA, 5×10^7 U/mg) was kindly provided by Dr. C. Dinarello (23). Recombinant human IFN-α (A/D) (SA, 7.9×10^7 U/mg) was generously provided by Drs. M. Brunda and P. Sorter of Hoffman-LaRoche (Nutley, NJ). Human IFN-α (A/D) hybrid has been shown to regulate mouse tissue in a manner similar to that of murine IFN-α (29). Indomethacin was purchased from Sigma (St. Louis, MO) and flurbiprofen from Cayman Chemical (Ann Arbor, MI).

Cell cultures. 3T3-F442A mouse embryo preadipocytes were generously provided by Dr. H. Green (Harvard Medical School). The cells were grown and differentiated as previously described (30) and used between 7-14 days after differentiation began. Differentiated adipocytes were incubated with cytokines in medium containing 2% bovine serum albumin, for 16 hours, unless otherwise indicated. Media was assayed for glycerol to measure lipolysis of stored triglyceride and for PGs. Cells were treated with heparin to release LPL for assay. In all experiments simultaneous controls were employed. None of the cytokines altered either protein or DNA content of the cultures.

Lipoprotein lipase assay. The cells were washed twice in Krebs-Ringer-Phosphate buffer, and incubated for 60 min at 37°C in 1 mL of buffer containing 4 U heparin/mL. An aliquot of buffer (0.9 mL) containing released LPL activity was incubated with 0.1 mL of a labeled triglyceride (^{3}H-triolein; New England Nuclear, Boston, MA) substrate (31) for 60 min. at 37°C. The reaction was terminated, free fatty acids extracted into 0.1 M KOH (32) and an aliquot counted in a scintillation counter.

Glycerol assay. A mixture of 0.5 mL medium and 0.5 mL 6% $HClO_4$ were centrifuged at 2000 rpm for 10 min. The acidified supernatant was neutralized by adding Freon and tri-n-octylamine for final ratio of 3:2:1.5 Freon:supernatant:tri-n-octylamine (33). The mixture was vortexed and centrifuged at 2000 rpm for 5 min. The upper phase was removed and the glycerol content determined by enzymatic fluorometric determination, as described (34).

Prostaglandin quantification. The supernatants (1 mL) were acidified with acetic acid and extracted three times with one mL ethyl acetate. The pooled extract was evaporated to dryness under nitrogen and PGs resuspended in phosphate buffered

saline containing 0.1% gelatin. Each sample was analyzed by radioimmunoassay, according to manufacturers instructions (Advanced Magnetics, Cambridge, MA), for PGE_2 and 6-keto $PGF_{1\alpha}$. Antiserum was obtained from Advanced Magnetics (Cambridge, MA), tritiated PGE_2 and 6-keto-$PGF_{1\alpha}$ from Du Pont (Wilmington, DE) and PGE_2 and 6-keto-$PGF_{1\alpha}$ standards from Cayman Chemical (Ann Arbor, MI).

Statistics. Significance was determined by using two-tailed Student's *t* test.

Results and Discussion

In a previous paper we reported that TNF stimulates lipolysis in 3T3-F442A adipocytes and that this effect of TNF is blocked by indomethacin, an inhibitor of PG synthesis (3). In the present study TNF (100 ng/mL) stimulated lipolysis (Figure 1A) and decreased LPL activity in the 3T3-F442A adipocytes (Figure 1B). Lipolysis was stimulated to 190% of control and LPL activity was decreased to 55% of control. As shown previously, indomethacin (100 μM) blocked the effect of TNF on lipolysis (Figure 1A). In addition, indomethacin blocked the effect of TNF on LPL activity (Figure 1B). Exogenous PGE_2 (500 ng/mL) stimulated lipolysis to 179% of control and decreased LPL activity to 56% of control. These results support the hypothesis that the effect of TNF on both lipolysis and LPL activity is mediated by PGE_2.

Increased PGE_2 production was first observed 6 h after incubation with TNF (Figure 2) and by 16 h the accumulation of PGE_2 was approximately 2 fold greater in the TNF treated cells than in the controls. 6-keto $PGF_{1\alpha}$ levels were increased 4 h after incubation with TNF and by 16 h the accumulation of 6-keto $PGF_{1\alpha}$ was 80% higher in

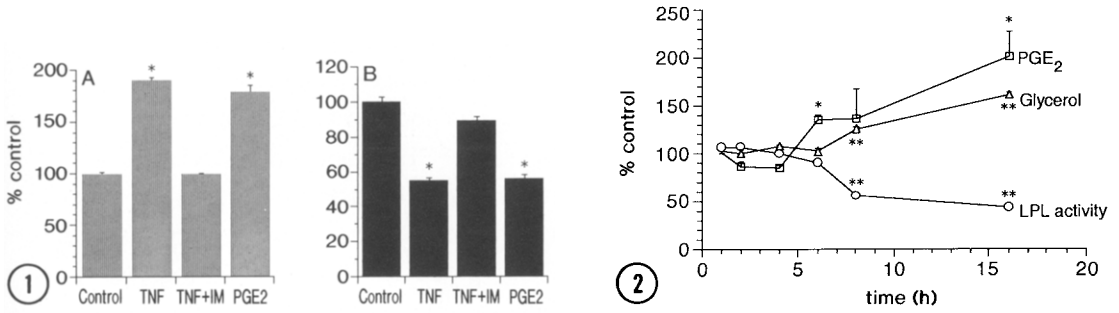

Figure 1. Effects of TNF, indomethacin and PGE_2 on lipolysis (A) and LPL activity (B) in 3T3-F422A adipocytes. Cells were incubated with medium only, 100 ng TNF/mL, 100 μM indomethacin + 100 ng TNF/mL or 500 ng PGE_2/mL for 16 h. The medium was removed and assayed for glycerol content to assess lipolysis as described in *Materials and Methods.* The cells were then washed and heparin-releasable LPL activity was measured as described in *Materials and Methods.* Data are mean ± SE (n=3). *P<0.001 *vs.* control.

Figure 2. Time course of TNF's effect on lipolysis, PGE_2 production and LPL activity in 3T3-F442A adipocytes. Cells were incubated with or without 100 ng TNF/mL. At the time points indicated, the medium was removed and assayed for glycerol content and PGE_2 as described in *Materials and Methods.* Heparin-releasable LPL activity was measured as described in *Materials and Methods.* Data are mean ± SE (n=3 for each data point). *P<0.05 *vs.* control; **P<0.001 *vs.* control.

Table 1
Effect of PGE_2 on lipolysis and LPL activity in 3T3-F442A adipocytes

Treatment	Glycerol mmoles/mL	LPL activity nmole FFA/mL/h
Control	0.61±0.01	226.3±4.7
TNF (100 ng/mL)	1.18±0.03*	105.8±1.4*
0.5 ng PGE_2/mL	0.64±0.01	248.7±7.8
5 ng PGE_2/mL	0.81±0.04*	246.6±6.9
50 ng PGE_2/mL	0.94±0.03*	237.8±2.9
500 ng PGE_2/mL	1.09±0.04*	187.5±3.1*

*Significantly different from control $P<0.05$ (n=3).
Values are means ± SE.

TNF treated cells than in controls (data not shown). Increased lipolysis and decreased LPL activity were first observed 8 h after incubation with TNF (Figure 2). By 16 h accumulation of glycerol was approximately 60% greater in the TNF-treated cells than in controls and LPL activity had decreased to 44% of that in controls. These results show that TNF's induction of PGs precedes its effect on lipolysis and LPL activity, which is consistent with the hypothesis that the effect of TNF might be mediated by PGs.

However, further investigation into the role of PG in TNF's action provided results that strongly suggest that TNF mediates its effect via a mechanism independent of PGs. The amount of PGs produced by the 3T3-F442A adipocytes following 16 h stimulation with TNF averaged 0.88 ng/mL for PGE_2 and 0.32 ng/mL for 6-keto $PGF_{1\alpha}$, compared to 0.41 ng/mL PGE_2 and 0.15 ng/mL 6-keto $PGF_{1\alpha}$ in controls. Incubations of cells with 0.5 ng/mL exogenous PGE_2 had no effect on lipolysis or LPL activity (Table 1). 5 ng/mL exogenous PGE_2 slightly enhanced lipolysis but did not affect LPL activity. Only at 500 ng/mL did PGE_2 have effects on lipolysis and LPL activity similar to that of TNF. These data indicate that the levels of PGE_2 produced by TNF are not sufficient to mediate the effects of TNF on lipolysis and LPL activity.

The dose response curve for indomethacin inhibition further revealed that 0.1, 1 or 10 μM indomethacin blocked TNF induced PGE_2 production without blocking TNF's effect on lipolysis or LPL activity (Table 2). Only at 100 μM did indomethacin block the effect of TNF on lipolysis and LPL activity. Moreover, flurbiprofen (1 μM), also blocked TNF stimulated PGE_2 production without blocking its effect on lipolysis or LPL activity (Table 2). These results demonstrate that TNF induced PG production is not required for the effect of TNF on lipolysis or LPL activity. The ability of higher concentrations of indomethacin to block TNF's effect on lipolysis and LPL activity is therefore independent of its effect on PG production.

We previously showed that indomethacin also blocks the effect of IL-1, IFN-α, IFN-β and IFN-γ on lipolysis (3). We therefore investigated whether these cytokines stimulate PG production by these adipocytes. IL-1 and IFN-α did not increase PG production

Table 2
Effect of TNF, indomethacin and flurbiprofen on lipolysis, LPL activity and PGE_2 production

Treatment	Glycerol mmoles/mL	LPL activity nmole FFA/mL/h	PGE_2 ng/mL
Control	0.88±0.01	204.6±6.3	0.57±0.15
TNF (100 ng/mL)	1.68±0.03*	73.2±4.2*	0.95±0.17
TNF + 0.1 μM indomethacin	1.69±0.01*	107.1±3.1*	0.50±0.34
TNF + 1 μM indomethacin	1.67±0.02*	123.3±5.5*	0.49±0.13
TNF + 10 μM indomethacin	1.54±0.03*	116.0±4.3*	0.46±0.04
TNF + 100 μM indomethacin	0.88±0.01	173.7±6.8*	o
Control	0.64±0.03	243.1±5.1	0.352±0.06
TNF (100 ng/mL)	1.16±0.02*	127.8±7.5*	1.001±0.25
TNF + 1 μM flurbiprofen	1.26±0.02*	125.6±2.5*	0.38±0.05

*Significantly different from control $P<0.05$ (n=3).
oindomethacin at this level interferes with the PG assay.
Values are means ± SEM.

(Table 3) while IFN-γ slightly increased the level of 6-keto $PGF_{1\alpha}$ (Table 3). These results demonstrate that the effect of these cytokines on lipolysis and LPL activity is also not dependent on PG production.

There are several alternative explanations for the ability of indomethacin to block cytokine action in 3T3 adipocytes. In addition to its effect on cyclooxygenase, indomethacin inhibits cyclic AMP-dependent protein kinase (35), phosphodiesterase (36) and at higher concentrations phospholipase A_2 (37). The ability of indomethacin to block cytokine induced stimulation of lipolysis and inhibition of LPL activity may be mediated through its action on one of these enzymes.

In summary, the present study demonstrates that cytokines stimulate lipolysis and decrease LPL activity via a PG independent mechanism. Because the cytokines

Table 3
The effect of IL-1, IFN-α and IFN-γ on lipolysis, LPL activity and PG production in 3T3-F442A adipocytes

Treatment	Glycerol mmoles/mL	LPL activity nmole FFA/mL/h	PGE_2 ng/mL	6-keto $PGF_{1\alpha}$ ng/mL
Control	1.06±0.03	213.14±6.39	0.18±0.04	0.11±0.01
IL-1 (100 ng/mL)	1.38±0.05*	150.46±3.22*	0.14±0.06	0.10±0.006
IFN-α (100 ng/mL)	1.59±0.06*	126.81±4.20*	0.20±0.04	0.11±0.01
IFN-γ (100 ng/mL)	1.55±0.05*	62.40±4.16*	0.29±0.06	0.33±0.08*

*Significantly different from control $P<0.05$ (n=3).
Values are means ± SEM.

studied here work through multiple different receptors, it is important to determine which common pathway of signalling is blocked by indomethacin.

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