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Research Article

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Biphasic Effects of Prostaglandin E_2 on the Human Fat Cell Adenylate Cyclase

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ABSTRACT Adenylate cyclase of human fat cell ghosts shows a biphasic response towards prostaglandin E_2 with inhibition occurring at nanomolar concentrations of the hormone and stimulation at concentrations beyond 10^{-6} mol/liter. The expression of the inhibitory effect is critically dependent on GTP. Under the conditions employed (1 mmol/liter ATP, 5 mmol/liter Mg^{2+}, 30°C) the inhibitory component of prostaglandin E_2 became apparent at GTP concentrations exceeding 10^{-6} mol/liter. The prostaglandin E_2-induced inhibition displayed characteristic features of prostaglandin action in intact fat cells with respect to the effective concentrations and degree of inhibition. It is concluded that prostaglandin E_2 is capable of inducing antagonistic effects upon lipolysis via interaction with the membrane-bound adenylate cyclase.

INTRODUCTION

Prostaglandins are among the most potent inhibitors of hormone-activated lipolysis in adipocytes of various species including man (1). Their inhibitory effect is associated with a decrease of intracellular 3',5'-cyclic AMP (cAMP).[1] Because prostaglandins are synthesized in response to lipolytic hormones (2, 3), it has been proposed that these C-20 fatty acids might act as feedback inhibitors of hormone-activated lipolysis at the level of the membrane-bound adenylate cyclase (4).

One of the major obstacles in elucidating the site of action of prostaglandins in fat cells is that their inhibitory effects have not yet been elicited in cell-free systems. In the rat, various types of prostaglandins had no direct effects on the adenylate cyclase of membrane preparations (1, 5). In other species, the direct effects of the prostaglandins were opposite to those to be expected from the negative feedback concept. We have recently shown that prostaglandins of the E and F type are stimulators of the human fat cell adenylate cyclase when tested over a concentration range of 10^{-6}–10^{-3} mol/liter (6). Gorman et al. (5) demonstrated that prostaglandin endoperoxide intermediates (0.28–28 μmol/liter) inhibited basal and noradrenaline stimulated adenylate cyclase from rat adipocyte ghosts. In intact cell preparations, however, these intermediates were rapidly converted to prostaglandin E_2 (PGE_2), which was physiologically more active, but had no direct effects on adenylate cyclase activity (7).

In this report, it is shown that the human fat cell adenylate cyclase exhibits biphasic responses to PGE_2, with inhibition occurring at submicromolar concentrations and stimulation at higher concentrations of the hormone.

METHODS

Biopsies of subcutaneous adipose tissue (10–15 g) were obtained from surgical patients not selected on the basis of age, sex, weight, or disease. The subjects were operated after an overnight fast. Anesthesia was induced with a short-acting barbiturate and maintained with halothane, nitrous oxide, and oxygen. The biopsies were usually obtained after the skin incision.

Experimental procedures were the same as described, (8). Fat cells and fat cell ghosts were prepared according to Rodbell (9). The adenylate cyclase activity was determined by the method of Salomon et al. (10) at 30°C. The assay mixture contained 25 mmol/liter Tris-HCl, pH 8.0, 5 mmol/liter $MgCl_2$, 20 mmol/liter creatine phosphate, 100 U/ml creatine phosphokinase, 1 mmol/liter cAMP, [α-^{32}P]ATP (1 mmol/liter; 40–50 cpm/pmol), and GTP as stated in the legends to figures and tables. The protein content of the samples was determined by the Lowry et al. (11) method. Statistical analysis was by the Wilcoxon test.

[α-^{32}P]ATP (9-11 Ci/mmol) and [^{3}H]cAMP (27 Ci/mmol) were purchased from the Radiochemical Centre Amersham, Bucks, England. Epinehrine-bitartrate and isoproterenol were from Merck AG, Darmstadt, West Germany. Enzymes, coenzymes and nucleotides were from Boehringer GmbH Mannheim, West Germany. PGE_2 was a gift of Dr. Brunnberg, Upjohn GmbH, Heppenheim, West Germany.

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[1] *Abbreviations used in this paper*: cAMP, 3',5'-cyclic AMP; PGE_2, prostaglandin E_2.

RESULTS

Fig. 1 shows a dose-response curve for PGE_2 over a wide range of concentrations (10^{-10} mol/liter–10^{-3}mol/liter) in the presence of 0.1 mmol/liter GTP. Under these conditions, the hormone displayed biphasic effects with inhibition occurring at 10^{-9}–10^{-7} mol/liter and stimulation at higher concentrations. The inhibitory effect was maximal at 2.8×10^{-8} mol/liter of PGE_2. At this concentration the hormome depressed basal enzyme activity by about 40%. The stimulatory effects of PGE_2 were detectable at concentrations beyond 2.8×10^{-7} mol/liter. The PGE_2-induced activation was much more impressive than the inhibitory effect. Activation was maximal at 2.8×10^{-4} mol/liter (five-fold increase of enzyme activity). Half-maximal effects occurred at a PGE_2-concentration of about 2.8×10^{-5} mol/liter.

Fig. 2 illustrates the inhibitory effects of two concentrations of PGE_2 on the dose-response curve for epinephrine. The GTP concentration was 0.1 mmol/liter. At concentrations of 10^{-9} mol/liter and 10^{-8} mol/liter the prostaglandin caused a dose-dependent and parallel rightward shift of the epinephrine dose-response curve, which has also been observed in metabolic experiments designed to investigate the antilipolytic effects of the E-type prostaglandins (12).

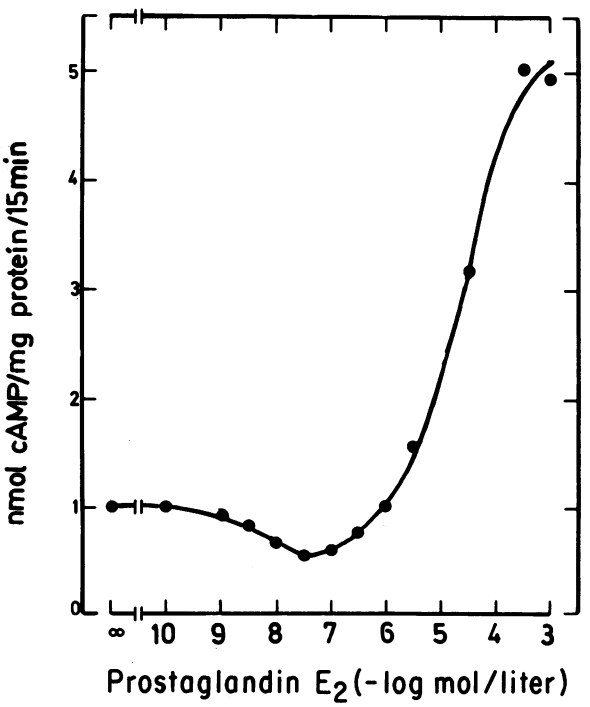

FIGURE 1 Dose-response curve for PGE_2. A representative experiment out of four is shown. Values are means of triplicate determinations.

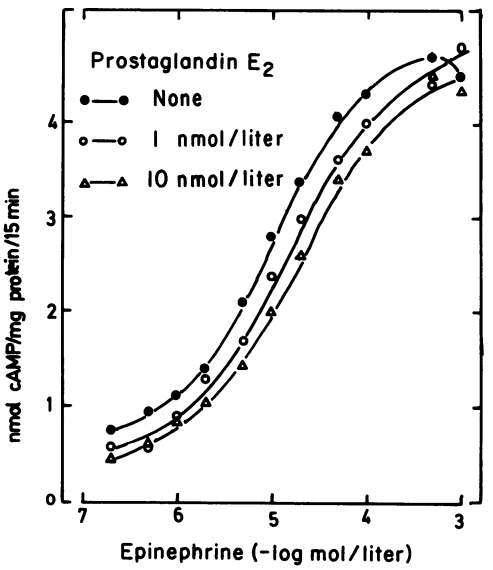

FIGURE 2 Effects of nanomolar concentrations of PGE_2 on the dose-response relationships of epinephrine. Values are means of triplicate determinations.

The PGE_2-induced inhibition was critically dependent on the presence of GTP in the assay medium. Fig. 3 shows the effect of the guanine nucleotide on the human fat cell adenylate cyclase activated by 10^{-5} mol/liter of isoproterenol in the absence and presence of 28 nmol/liter of PGE_2. Increasing concentrations of GTP induced a biphasic response of the isoproterenol-activated enzyme. Peak activity occurred at a GTP concentration of $\cong 10^{-4}$ mol/liter, whereas higher concentrations of the nucleotide depressed the rate of

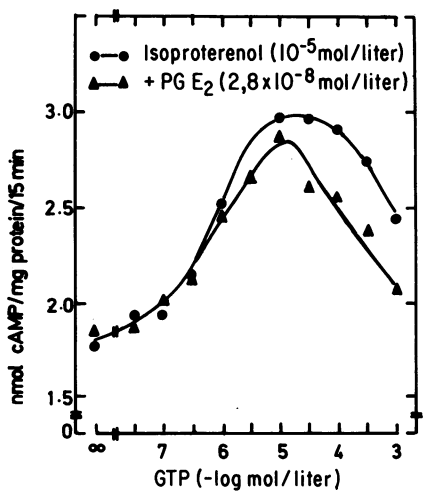

FIGURE 3 GTP-dependence of the PGE_2-Induced inhibition of the human fat cell adenylate cyclase. A representative experiment out of four is shown. Values are means of triplicate determinations.

cAMP formation relative to peak activity. PGE_2 had no effect at GTP concentrations below 10^{-6} mol/liter. Inhibition was most pronounced at GTP concentrations beyond 10^{-5} mol/liter.

The inhibitory effects of nanomolar concentrations of PGE_2 were confirmed in six separate experiments carried out with membranes from different subjects. The catecholamine concentrations were chosen such as to elicit submaximal activation. PGE_2 caused a significant inhibition ($P \leqq 0.05$) by about 25% in these experiments. Quantitatively and qualitatively similar results were obtained with either isoproterenol or the naturally occurring catecholamine epinephrine (Table I).

DISCUSSION

The inhibition of the human fat cell adenylate cyclase demonstrated in this report reflects the characteristic features of the antilipolytic effects of the E-prostaglandins in intact fat cells in many aspects. The effects of micromolar concentrations of lipolytic hormones can be inhibited by nanomolar concentrations of the E-type prostaglandins, making this class of hormone's orders of magnitude more potent than agents like nicotinic acid or propranolol (1). The PGE_2 concentrations inducing an inhibition of the human fat cell enzyme are in the physiological range. In addition, the maximal inhibition of hormone-stimulated lipolysis because of E-prostaglandins is only about 50% (12). This applies to the depression of the activity of the human fat cell adenylate cyclase by physiological concentrations of PGE_2 too. The prostaglandins share this latter property, i.e., incomplete inhibition of lipolysis, in common with adenosine (1). Interestingly, adenosine analogues, in a very recent report, have been shown to induce an inhibition of isoproterenol-stimulated rat fat cell adenylate cyclase that was also GTP-dependent (13).

TABLE I

Inhibition of the Catecholamine-Activated Adenylate Cyclase by Prostaglandin E_2 (2.8×10^{-8} mol/liter)

Additions	Adenylate cyclase activity*	
	Control‡	PGE_2 (28 nmol/liter)‡
	nmol cAMP/mg protein/15 min	
Epinephrine (5×10^{-5} mol/liter)	1.7±0.2	1.3±0.2§
Isoproterenol (1×10^{-5} mol/liter)	2.4±0.3	1.9±0.2§

* The assay mixture contained 0.1 mmol/liter GTP.

‡ Values are mean±SEM of six separate experiments carried out in triplicate.

§ Significantly lower ($P \leqq 0.05$) than the corresponding controls.

The main effect of the prostaglandins appears to be an inhibitory one on the mobilization of free fatty acids and glycerol from adipose tissue. However, these C-20 fatty acids can also stimulate lipolysis under certain conditions suggesting that not only inhibition but also stimulation of the human fat cell adenylate cyclase is of physiological importance in human fat cell function (14, 15). Our results are thus compatible with the concept that the antagonistic effects of the E-type prostaglandins on lipolysis are mediated via interaction with the membrane-bound adenylate cyclase thereby suggesting that the role of these hormones is more complex than assumed by the negative feedback concept. This hypothesis considers only an inhibition of adenylate cyclase but does not take into account the biphasic prostaglandin effects shown in this report. In contrast to the negative feedback concept our results, therefore, support the contention that the actual effects of prostaglandins are the result of a delicately regulated balance between two opposing prostaglandin effects, which is not only dependent on the concentration of these C-20 fatty acids but is also extremely sensitive to modulation by guanine nucleotides.

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