JCI The Journal of Clinical Investigation

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J Clin Invest. 1964;43(8):1533-1540. https://doi.org/10.1172/JCI105030.

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Journal of Clinical Investigation
Vol. 43, No. 8, 1964

Effects of the Prostaglandins on Hormone-induced Mobilization of Free Fatty Acids *

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About 30 years ago, Goldblatt (1, 2) and von Euler (3, 4) showed that human and ovine seminal plasma contain an acidic lipid (prostaglandin) with vasodepressor activity and smooth muscle-stimulating activity. The early studies have been summarized by von Euler (5, 6). In 1960 Bergström and Sjövall isolated from sheep vesicular glands a highly potent crystalline material, prostaglandin E_1 (PGE_1) (7).[1] The structure of this compound (Figure 1) was established as 2 (6-carboxyhexyl)-3-(3-hydroxyocten-1-yl)-4-hydroxycylopentanone (8) or 11α, 15-dihydroxy-9-ketoprost-13-enoic acid. Further studies showed that two closely related active compounds, PGE_2 and PGE_3, differing in having one and two additional double bonds, respectively, are also present in sheep vesicular glands (9). Human semen contains approximately equal amounts of these three prostaglandins (10), and PGE_1 has been demonstrated in the calf thymus (11). The corresponding compounds in which the keto group of the E compounds has been reduced to an alcohol group ($PGF_{1\alpha}$, $PGF_{2\alpha}$, and $PGF_{3\alpha}$) have been isolated in small amounts from seminal plasma, and $PGF_{2\alpha}$ has been isolated from lungs of sheep and pig (12) and from sheep iris (13).

The high concentration of prostaglandin in seminal plasma suggested that its primary role would be in relation to sexual function, perhaps facilitating passage of sperm up the genital tract by relaxing the uterus (5). On the other hand, the presence of prostaglandins has now been established in a variety of other tissues. Whereas the concentrations found are very low, the compounds are extremely potent vasodepressor agents in man and in experimental animals (6, 14), and a more general physiological function for these unusual compounds remains a possibility.

The present studies show that very low concentrations of PGE_1 inhibit glycerol production in adipose tissue and counteract the stimulation of glycerol release induced by catecholamines, glucagon, ACTH, and TSH. This is shown to be due to an interference with the activation of tissue lipase usually produced by exposure of adipose tissue to these hormones. The relative potencies of the several prostaglandins is compared. The vasopressor effects of epinephrine or norepinephrine in dogs are also shown to be blocked by PGE_1. A preliminary report of this work has appeared elsewhere (15).

Methods

The methods and materials used in the *in vitro* studies have been described in previous papers (16, 17). Briefly, epididymal fat pads were taken from Sprague-Dawley rats (150 to 200 g) fed ad libitum until killed by decapitation. Tissues were incubated 1 hour at 37° C under 95% O_2 and 5% CO_2 in 3 ml Krebs' bicarbonate medium containing bovine serum albumin (30 mg per ml). Glucose was not added to the medium. Glycerol was determined by Korn's modification (18) of the method of Lambert and Neish (19). FFA were determined by Dole's method (20), using isooctane in place of heptane.

Tissue phosphorylase was assayed in homogenates prepared at the end of the incubation by methods previously described (21). Lipase activity in the same homogenates was assayed by methods shown in previous studies to maximize the effects of epinephrine (22, 23). The tissue was homogenized in 10 vol of 0.154 M KCl, and suitable samples were added to an incubation mixture containing 30 mg bovine serum albumin and 20 μmoles of sodium phosphate buffer, pH 7.0, in a final volume of 1.0

* Submitted for publication September 23, 1963; accepted March 19, 1964.

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[1] Abbreviations used: PG = prostaglandin (PGE_1 = prostaglandin E_1, PGE_2 = prostaglandin E_2, etc.) and TSH = thyroid-stimulating hormone.

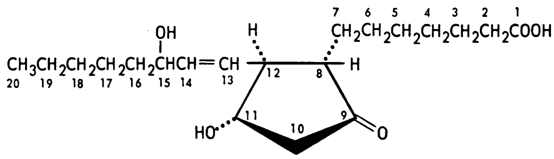

FIG. 1. STRUCTURE OF PGE_1. Reduction of the keto group at position 9 yields PGF_1, which can exist in two stereoisomeric forms—$PGF_{1\alpha}$ and $PGF_{1\beta}$. PGE_2 differs from PGE_1 only in having an additional double bond at the 5,6 position; PGE_3 has an additional double bond at 5,6 and at 17,18. PGF_2 and PGF_3 are the 9-hydroxy compounds analogous to PGF_1.

ml. The reaction was stopped by addition of 1 ml of a mixture containing ethanolamine (0.9 M), acetic acid (0.1 M), and Cu $(NO_3)_2 \cdot 3\ H_2O$ (5%). The copper salts of the fatty acids were extracted into chloroform, and the amount of copper present in the chloroform was determined colorimetrically. This procedure, adapted from the method described by Duncombe (24), was described in detail in an earlier publication from this laboratory (23).

Mongrel dogs were anesthetized with intravenous pentobarbital. Mean femoral arterial pressure was monitored continuously with a Statham strain gauge. Catecholamines and prostaglandins were administered by a femoral vein catheter, and blood samples for analysis were drawn from a catheter in the opposite femoral artery advanced into the aorta. Blood glucose was determined with glucose oxidase.[2] Plasma FFA were determined by Dole's method (20).

The prostaglandin preparations were fully characterized crystalline compounds isolated from sheep prostate glands and, in some cases, chemically modified as described elsewhere (7–9). Stock solutions in ethanol were stored at − 10° C and diluted in saline or in buffers before use. The final incubation medium contained 0.002% ethanol both in control and experimental flasks. The structure of PGE_1 is shown in Figure 1. PGE_2 differs from it only in having an additional double bond in the 5,6 position, whereas PGE_3 has additional double bonds at both the 5,6 and the 17,18 positions. The compounds of the F series are analogous to those of the E series except that the 9-keto group has been reduced to a hydroxyl group. The suffixes α and β denote the stereoisomeric configuration of this hydroxyl group.

Results

In vitro release of glycerol from fat pad. As shown in Table I, PGE_1 alone (0.1 μg per ml) significantly reduced basal glycerol release from adipose tissue. At this same low concentration it markedly reduced the stimulating effects of epinephrine, norepinephrine, glucagon, and ACTH. The effect of TSH was not significantly inhibited when PGE_1 was added only at the start of the 1-hour incubation, as in the other studies. When, however, a second addition of 0.1 μg PGE_1 per ml was made after 30 minutes of incubation, there was a significant suppression of the TSH effect.

PGE_2 was also effective in counteracting the epinephrine stimulation of glycerol release, but appeared to be less effective than PGE_1 (Table II). Because of the considerable variation in response to hormone stimulation observed in tissues from different rats, it is difficult to compare the potency of inhibitors studied in separate experiments. A direct comparison of potencies was made by incubating both fat pads from a single rat with the lipolytic hormone and adding PGE_1 to one flask, PGE_2 to the other. The results of this direct comparison, shown in Table III, indicated that PGE_2 was less potent than PGE_1 in antagonizing the action of ACTH and glucagon. The difference in the case of the epinephrine experiments was of borderline significance.

PGE_3 at 0.1 μg per ml did not suppress the action of epinephrine, ACTH, or glucagon (Table II). At 10 times this concentration, there was still no inhibition in the presence of epinephrine. A very high concentration of PGE_3, 16.6 μg per ml, significantly suppressed the activity of epinephrine present at a concentration of 0.1 μg per ml.

TABLE I
*Effects of prostaglandin E_1 (PGE_1) on hormone-stimulated glycerol release from rat epididymal fat pads**

Hormone added	Glycerol release: Without PGE_1	Glycerol release: Δ due to PGE_1†	p value
		µmoles/g/hr	
None‡	0.9	−0.3 ± 0.06	<0.001
Epinephrine, 0.1 µg/ml	3.1	−1.5 ± 0.38	<0.01
Norepinephrine, 0.2 µg/ml	5.5	−2.2 ± 0.13	<0.001
Glucagon, 5 µg/ml	3.1	−1.6 ± 0.27	<0.002
ACTH, 0.04 U/ml	4.6	−1.6 ± 0.21	<0.001
TSH, 10 µg/ml§	3.0	−0.3 ± 0.2	NS
TSH, 10 µg/ml‖	4.7	−1.5 ± 0.27	<0.005

* Six pairs of tissues in each hormone study were incubated for 1 hour in 3 ml Krebs' bicarbonate medium containing bovine serum albumin, 30 mg per ml, in an atmosphere of 95% oxygen and 5% carbon dioxide. Hormones were added to both flasks; PGE_1 (0.1 µg per ml) to only one.
† Mean of differences between paired tissues ± standard error of the mean.
‡ Data from 16 pairs of tissues.
§ TSH = thyroid-stimulating hormone.
‖ PGE_1, 0.1 µg per ml, added at zero time and again after 30 minutes.

[2] Glucostat reagents, Worthington Biochemical Corp., Freehold, N. J.

TABLE II

*Effects of PGE_2, $PGF_{2\alpha}$, and PGE_3 on hormone-stimulated glycerol release from fat pad**

Lipolytic hormone added	Prostaglandin compound added	Glycerol release: Without PG compound	Glycerol release: Δ due to PG compound†	p value
		μmoles/g/hr		
Epinephrine, 0.1 μg/ml	PGE_2, 0.1 μg/ml (6)‡	4.4	−1.8 ± 0.78	<0.1
Epinephrine, 0.1 μg/ml	PGE_2, 0.5 μg/ml (10)	4.12	−0.80 ± 0.13	<0.001
Epinephrine, 0.1 μg/ml	$PGF_{2\alpha}$, 0.5 μg/ml (12)	4.14	−0.37 ± 0.18	NS
Epinephrine, 0.1 μg/ml	PGE_2, 5.0 μg/ml (6)	4.02	−1.87 ± 0.26	<0.001
Epinephrine, 0.1 μg/ml	$PGF_{2\alpha}$, 5.0 μg/ml (6)	3.52	−1.42 ± 0.31	<0.01
Epinephrine, 0.1 μg/ml	PGE_3, 0.1 μg/ml (6)	4.62	−0.01 ± 0.20	NS
Epinephrine, 0.1 μg/ml	PGE_3, 1.0 μg/ml (6)	3.62	−0.45 ± 0.20	NS
Epinephrine, 0.1 μg/ml	PGE_3, 16.6 μg/ml (6)	2.40	−0.91 ± 0.20	<0.01
ACTH, 0.04 U/ml	PGE_2, 0.1 μg/ml (6)	4.6	−0.2 ± 0.3	NS
ACTH, 0.04 U/ml	PGE_3, 0.1 μg/ml (6)	4.67	−0.72 ± 0.57	NS
Glucagon, 5 μg/ml	PGE_3, 0.1 μg/ml (6)	1.31	−0115 ± 0.16	NS

* Incubations were carried out as described in the footnote for Table I.
† Mean of differences between paired tissues ± standard error of the mean.
‡ The number of pairs of tissues in each group is indicated in parentheses.

The activities of PGE_1 and its derivatives, $PGF_{1\alpha}$ and $PGF_{1\beta}$, are compared in Table IV. At 0.5 μg per ml the F derivatives had no significant effects on epinephrine-induced glycerol release. At 5 μg per ml, $PGF_{1\alpha}$ had a small effect, but $PGF_{1\beta}$ was still inactive.

The activities of PGE_2 and its derivative $PGF_{2\alpha}$ can be compared in Table II. At a concentration of 0.5 μg per ml, PGE_2 had a highly significant effect in the presence of epinephrine, whereas $PGF_{2\alpha}$ was ineffective. At a tenfold higher concentration $PGF_{2\alpha}$ had a significant suppressive effect. A direct comparison of PGE_2 and $PGF_{2\alpha}$ in paired tissues, both exposed to epinephrine as discussed above, showed that the F derivative was distinctly less potent (Table III).

In many of the studies summarized above, FFA release into the medium was also measured. The

TABLE III

*Direct comparison of inhibiting activities of PG compounds on hormone-stimulated glycerol release**

Hormone added in both flasks	PG compound in flask A	PG compound in flask B	Difference in glycerol release† A−B	p value
			μmole/g/hr	
Epinephrine, 0.1 μg/ml	PGE_1, 0.1 μg/ml	PGE_2, 0.1 μg/ml	−0.9 ± 0.4	<0.1
ACTH, 0.04 U/ml	PGE_1, 0.1 μg/ml	PGE_2, 0.1 μg/ml	−0.4 ± 0.12	<0.02
Glucagon, 5 μg/ml	PGE_1, 0.1 μg/ml	PGE_2, 0.1 μg/ml	−1.3 ± 0.17	<0.001
Epinephrine, 0.1 μg/ml	PGE_2, 2 μg/ml	$PGF_{2\alpha}$, 2 μg/ml	−0.73 ± 0.25	<0.05

* Six pairs of fat pads in each experiment. Hormones were added at concentrations indicated to both flasks. Prostaglandin compounds to be compared for potency were added respectively to flask A and to flask B. A negative value (A−B) indicates that the prostaglandin compound in flask A more effectively blocked glycerol production.
† Mean of differences between paired tissues ± standard error of the mean.

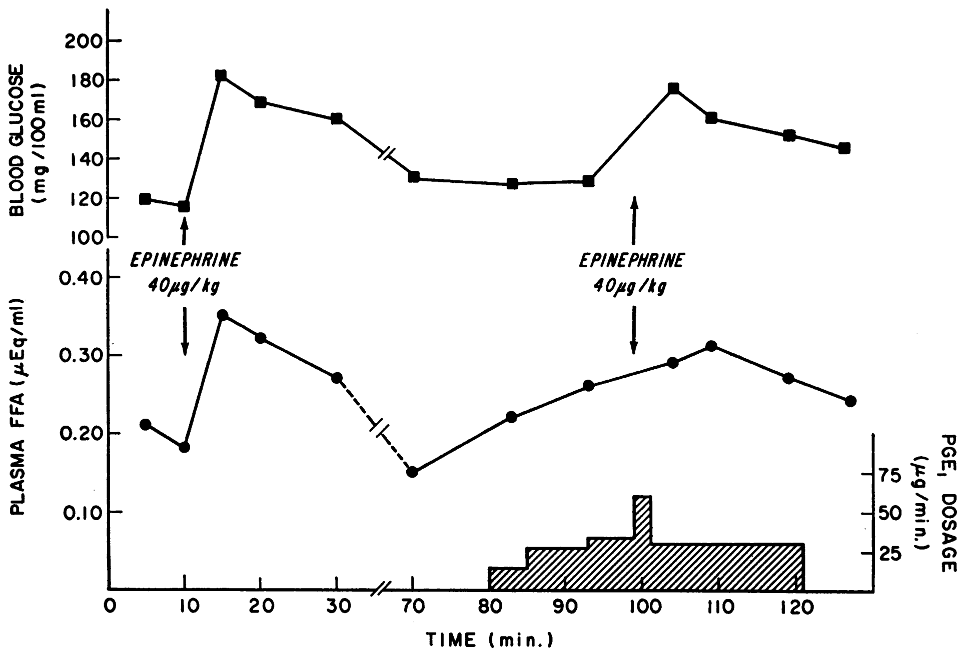

FIG. 2. CHANGES IN BLOOD GLUCOSE AND PLASMA FFA LEVELS INDUCED IN A 15.4-KG DOG BY EPINEPHRINE IN A CONTROL PERIOD AND DURING INFUSION OF PGE_1 (indicated by cross-hatched area).

results generally paralleled closely those obtained for glycerol. An important exception was encountered in the studies of the effect of PGE_1 in the absence of any added lipolytic hormones. Here there was no significant effect on basal FFA release even though, as shown in Table I, a significant suppression of basal glycerol release could be demonstrated.

TABLE IV
*Comparison of inhibitory activities of PGE_1 and of its PGF derivatives on epinephrine-stimulated glycerol release**

Concentration of prostaglandin derivative	Glycerol release: Without PG	Glycerol release: Δ release due to PG†	p value
		μmoles/g/hr	
PGE_1, 0.5 μg/ml	1.83	-1.13 ± 0.16	<0.01
$PGF_{1\alpha}$, 0.5 μg/ml	1.89	-0.37 ± 0.41	NS
$PGF_{1\beta}$, 0.5 μg/ml	2.16	-0.62 ± 0.69	NS
PGE_1, 5 μg/ml	6.76	-3.55 ± 0.42	<0.005
$PGF_{1\alpha}$, 5 μg/ml	3.97	-0.68 ± 0.21	>0.05
$PGF_{1\beta}$, 5 μg/ml	3.80	$+0.04 \pm 0.29$	NS

* Four pairs of fat pads in each experiment. All flasks contained epinephrine, 0.1 μg per ml. Experimental flasks contained in addition the indicated prostaglandin derivative.
† Mean of differences between paired tissues ± standard error of the mean.

Changes in tissue lipase and phosphorylase activity. Vaughan has shown that epinephrine and several other lipolytic hormones can activate adipose tissue phosphorylase (21). These hormones have also been shown to activate lipase system in adipose tissue (22, 23, 25–27). With methods described previously (23), it was shown that the degree of lipase activation induced by epinephrine was decreased by adding PGE_1 along with the epinephrine (Table V). The activation of phosphorylase in these same experiments was also significantly reduced. In these studies, the PGE_1 effect was evaluated directly by using paired tissues both of which were exposed to epinephrine. The magnitude of the epinephrine-induced enzyme activation can therefore be estimated only by reference to other similar studies (21, 23). Such a comparison suggests that the effect on phosphorylase activation was smaller than that on lipase activation.

In vivo studies in anesthetized dogs. Intravenous injection of epinephrine (40 μg per kg) into an anesthetized dog caused the expected rise in plasma FFA and blood glucose levels (Figure 2). When the same dose of epinephrine was repeated while maintaining an intravenous infusion

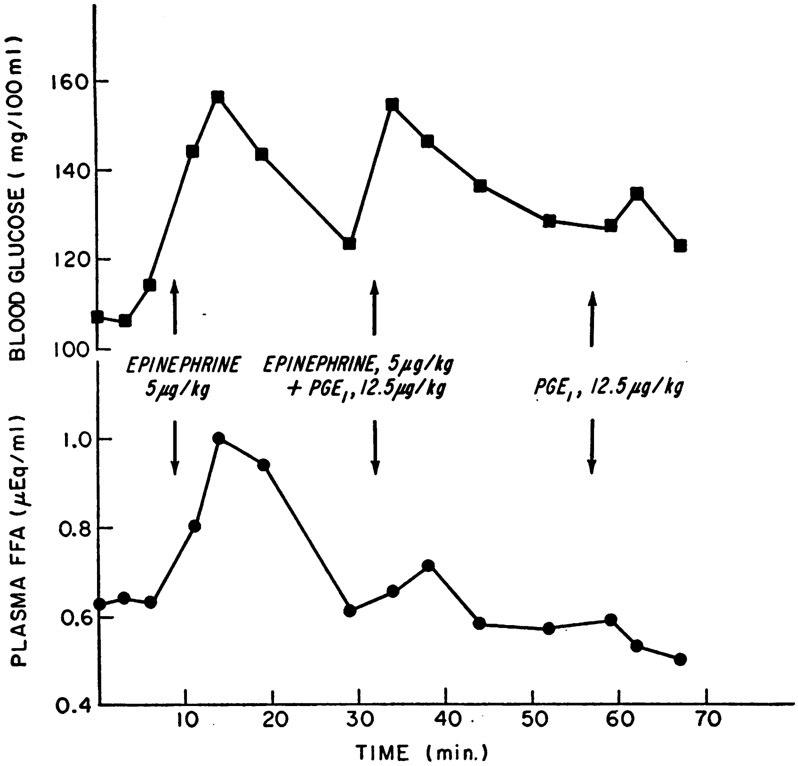

FIG. 3. CHANGES IN BLOOD GLUCOSE AND PLASMA FFA LEVELS INDUCED IN A 13 KG DOG BY INJECTION OF EPINEPHRINE ALONE, BY SIMULTANEOUS INJECTION OF EPINEPHRINE PLUS PGE_1, AND BY PGE_1 ALONE, IN THE DOSAGES INDICATED.

of PGE_1 at the rates shown in Figure 2, the peak of the glucose response was comparable to that observed initially, but there was little or no change in plasma FFA.

Figure 3 shows the responses to a single intravenous injection of epinephrine, 5 μg per kg, given alone and then repeated with 12.5 μg per kg of PGE_1 injected simultaneously. Again the glucose response was apparently not altered while the FFA response was all but abolished. Injection of PGE_1 alone in this case was followed by a slight fall in the FFA level, but no definite change in blood glucose level.

Intravenous injection of PGE_1 alone, 12.5 μg per kg, caused a prompt fall in mean femoral arterial pressure from 100 to 60 mm Hg, with a return to control level by 2 minutes. In the same animal, epinephrine alone elevated mean pressure from 100 to approximately 180 mm Hg. When both compounds were simultaneously administered at these same dosages, the pressure rose to 150 mm Hg. A number of such studies were performed at various dose ratios of prostaglandin to epinephrine. At ratios of 2 or 3 to 1, PGE_1 consistently reduced and sometimes abolished the pressor response to epinephrine. At higher ratios there was a transient rise in mean pressure, per-

TABLE V

*Effects of PGE_1 on epinephrine-induced changes in adipose tissue lipase and phosphorylase activities**

	Without PGE	PGE effect†
Lipase activity, μEq FFA/g/20 min	5.1	−2.0 ± 0.58
Phosphorylase activity, μmoles P/g/30 min	48.6	−3.5 ± 0.73

* Paired epididymal fat pads were incubated for 2 hours in 3 ml Krebs' bicarbonate buffer containing 3% bovine serum albumin. Three minutes before the end of the incubation, epinephrine was added to one flask (0.3 to 0.7 μg per ml), and both epinephrine and an equal weight of PGE_1 were added to the paired flask. The tissues were homogenized and lipase activity and phosphorylase activity were assayed as described previously (19, 21).

† Results of 11 experiments, expressed as a mean of differences between pairs ± standard error of the mean.

sisting for less than 30 seconds, followed by a fall in pressure returning to control values over the course of the next 1 to 2 minutes.

Discussion

The studies reported above show that PGE_1 is a remarkably potent antagonist of the fat-mobilizing action of catecholamines *in vitro*. At 2.8×10^{-7} M concentration, it was highly effective in suppressing the action of norepinephrine present at twice that molar concentration. Significant inhibition was observed at PGE_1 concentrations as low as 5.6×10^{-8} M. The lipolytic action of glucagon, ACTH, and TSH was also inhibited. The fact that less than stoichiometric amounts of PGE_1 inhibited effectively and the fact that it inhibited the action of these several lipolytic hormones of differing molecular structure suggests that it does not act simply through complex formation with the hormones. The intimate mechanism by which the fat-mobilizing hormones lead to activation of the hormone-sensitive lipase in adipose tissue is not known. The most that can be said is that prostaglandin must interfere at some point in the pathway common to the several hormones whose activity it blocks.

Although the effect of PGE_1 on hormone-stimulated activation of phosphorylase appears to be smaller in percentage terms than its effect on lipase activation, this direct quantitative comparison must not be considered definitive, since the absolute magnitude of the hormone-induced increments in enzyme activity, relative to control tissue, was not determined. Preliminary studies with rat liver slices failed to reveal any effect of PGE_1 on epinephrine stimulation of glucose release.

Direct comparison of the potency of the different PGE compounds showed that PGE_2, although it was active at relatively low concentrations (0.5 to 5.0 μg per ml), was less effective than PGE_1 in blocking hormone-stimulated lipolysis. PGE_3 was without effect at concentrations up to 1 μg per ml; at 16.6 μg per ml it significantly reduced epinephrine-stimulated lipolysis. It can be concluded that all three PGE compounds are active, with potencies in the order $PGE_1 > PGE_2 > PGE_3$.

$PGF_{1\alpha}$ and $PGF_{1\beta}$, 0.5 μg per ml, did not significantly inhibit epinephrine-stimulated lipolysis. $PGF_{1\alpha}$, 5 μg per ml, caused some inhibition, but $PGF_{1\beta}$ was still without effect at this higher concentration. The relative potencies appear to be $PGE_1 > PGF_{1\alpha} > PGF_{1\beta}$. $PGF_{2\alpha}$ was without significant effect at 0.5 μg per ml, but was active at a concentration of 5 μg per ml. Direct comparison confirmed that PGE_2 was more potent than its F derivative, $PGF_{2\alpha}$.

The studies in dogs demonstrate the ability of PGE_1 to suppress mobilization of FFA induced by epinephrine *in vivo*. There was, however, no apparent effect on the degree of hyperglycemia produced by epinephrine. Bergström, Carlson, and Orö (28) have recently studied the effects of PGE_1 *in vivo* more extensively, making use of constant infusion techniques. Their results confirm the ability of PGE_1 to block the FFA-mobilizing action of catecholamines. The availability of these potent inhibitors may be of help in further elucidation of mechanisms controlling fat mobilization. Catecholamines, ACTH, TSH, glucagon, and vasopressin increase the rate of lipolysis in adipose tissue, apparently by favoring the conversion of a specific hormone-sensitive lipase from an inactive to an active form (22, 23, 25–27, 29). All of these hormones similarly bring about an increase in phosphorylase activity in adipose tissue. The effects on phosphorylase activity are presumably mediated by cyclic 3′,5′-adenosine monophosphate (AMP), but the role of this nucleotide in the lipase system remains to be determined. Cyclic AMP is an intermediate in the effects of vasopressin on permeability of the toad bladder, and it has recently been shown that PGE inhibits the action of vasopressin in that tissue (30) just as it inhibits the effect of vasopressin on lipolysis in adipose tissue (29).

The potent vasodepressor action of PGE_1 was confirmed (6, 14), and it was further shown that PGE_1 sharply reduces the pressor effect of equimolar amounts of epinephrine and of norepinephrine when injected intravenously at the same time as the catecholamines. Since PGE_1 alone in the dosages used is strongly vasodepressor, whether it interferes with the hormone-activated mechanism leading to contraction of smooth muscle or whether it simply causes vasodilation by an independent mechanism cannot be decided.

Summary

Prostaglandin E_1 (PGE_1) at 2.8×10^{-7} M concentration effectively counteracted the fat-mobilizing activities of epinephrine, norepinephrine, adrenocorticotropic hormone, glucagon, and thyroid-stimulating hormone on the rat epididymal fat pad *in vitro,* measured in terms of glycerol release into the medium. In the absence of added hormones, the rate of glycerol release from fat pads was slightly decreased by PGE_1. PGE_1 interfered with the epinephrine-induced activation of a hormone-sensitive lipase in adipose tissue. There was also a small but significant interference with epinephrine-induced activation of phosphorylase.

The relative potencies of a series of closely related compounds in the prostaglandin family as inhibitors of epinephrine-induced fat mobilization *in vitro* were determined.

The high potency of PGE_1 as a vasodepressor agent was confirmed. When injected intravenously into dogs along with approximately equimolar amounts of epinephrine or norepinephrine, PGE_1 counteracted the pressor activity of the catecholamines as well as the rise in plasma free fatty acids normally produced. The hyperglycemic response to epinephrine did not appear to be altered.

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