Effects of prostaglandin E_1 on lipolysis and plasma free fatty acids in the fasted rat

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ABSTRACT Contrary to published reports, prostaglandin E_1 (PGE_1) in vitro and in vivo inhibited fasting lipolysis in rats. Adipose tissue lipolysis was inhibited when the tissue was incubated in the presence of PGE_1 and when the compound was administered intravenously. A biphasic plasma free fatty acid (FFA) response was obtained in fasted rats after intravenous injection of 80 μg of PGE_1 per kg body weight; plasma FFA concentrations were lowered at 7 min, elevated at 15 min, and at normal concentrations at 30 min. The FFA depression at 7 min was independent of the animal's nutritional state, but the rebound at 15 min did not occur in fed rats. The plasma FFA rebound in fasted rats at 15 min may be a consequence of rapid inactivation of PGE_1, followed by unopposed activity of factors which enhance fasting lipolysis.

KEY WORDS *prostaglandin* E_1 · *rat* · lipolysis · plasma free fatty acids · adipose tissue

PROSTAGLANDINS are vasodepressor and smooth muscle-stimulating lipids originally reported to be present in seminal fluid (1–4). Recently, Bergström and collaborators isolated and characterized a number of prostaglandins (5, 6), several of which were shown to inhibit both baseline and hormonally-induced lipolysis in rat and human adipose tissue in vitro (7–9). One compound of this series, prostaglandin E_1 (PGE_1), also inhibited the fat-mobilizing effect of epinephrine in anesthetized (10, 11) and unanesthetized dogs (11–13). The possibility that the nutritional state influences the effect of PGE_1 was indicated in reports from several laboratories. For example, PGE_1 in vitro did not affect adipose tissue lipolysis in rats fasted for 20 and 44 hr (14, 15); however, it inhibited glycerol and FFA release from epididymal adipose tissue of normal fed rats as well as of alloxan-diabetic rats (15). PGE_1 in vivo inhibited the fat-mobilizing effect of cold exposure, ACTH, and norepinephrine, but not of a 36 hr fast in rats (16). Other studies suggest a difference between fasting and hormonally induced lipolysis, for example hypophysectomized animals mobilize fat in response to fasting but not to epinephrine (17, 18). These reports prompted this investigation, which demonstrates that PGE_1 lowers plasma FFA and glycerol in both fasted and fed rats and also inhibits lipolysis in vitro in epididymal adipose tissue of fasted rats.

Abbreviations: PGE_1, prostaglandin E_1; FFA, free fatty acid(s).

MATERIALS AND METHODS

Male Spartan rats (Spartan Research, Haslett, Mich.) weighing 240–260 g before fasting were used. A 0.2% ethanol solution of crystalline PGE_1 either was diluted at least 100-fold with saline or was evaporated to dryness under N_2, the resultant PGE_1 being suspended in saline with the aid of a Potter-Elvehjem homogenizer. 1 ml of either preparation was injected into a tail vein.

Most animals were anesthetized with ether and bled from the abdominal aorta into syringes coated internally with a 1% heparin solution. Animals in some of the experiments described in Table 4 were decapitated. Fragments of epididymal adipose tissue (50–60 mg) were incubated in duplicate in 1 ml of Krebs-Ringer bicarbonate or phosphate medium (as indicated) containing 3% crystalline bovine albumin (Armour) and 0.9 mg glucose. The tissue system was incubated in Potter-Elvehjem, all-glass homogenizer tubes at 37°C in an atmosphere of 5% CO_2 and 95% air in a Dubnoff metabolic shaker, oscillating at 60–70 cycles/min. After 2 hr of incubation, adipose tissue was homogenized in the medium. Aliquots of homogenate and of plasma were analyzed for FFA concentration by the procedure of Dole (19) as modified by Ko and Royer (20). Glycerol concentration of adipose tissue and plasma was measured

by the fluorometric method of Chernick and Reiter (personal communication).

RESULTS

In preliminary studies with fed rats and with rats fasted for 20 hr, PGE_1 had no effect on plasma FFA concentrations 1 hr after injection. This unexpected result in fed rats led to experiments in which plasma FFA concentrations were measued at earlier time intervals, since some other biological effects of PGE_1, for example lowering of blood pressure, are known to be transient (21, 22). Furthermore, 15 min after subcutaneous administration of 0.2 μg of PGE_1, Samuelsson found no unchanged compound in the plasma of rats (23).

Table 1 shows the response of plasma FFA 7 and 15 min after injection of various doses of PGE_1 in rats fasted for 20 hr. 7 min after injection plasma FFA concentration was depressed at all doses greater than 16 μg/kg. The decrease was maximal with 80 μg/kg and was not changed by higher doses of PGE_1. After 15 min plasma FFA were elevated at all doses and reached a maximum with a dose of 80 μg/kg. The data in Table 2 show that elasma FFA return to control levels 30 min after inpction of PGE_1.

Table 3 shows that the FFA content of nonincubated adipose tissue taken from rats 7 min after injection with PGE_1 was significantly lower than in control animals and suggests that lowering of plasma FFA is a reflection of reduced lipolysis in adipose tissue.

TABLE 1 PLASMA FFA CONCENTRATION OF FASTED RATS 7 AND 15 MIN AFTER INJECTION OF VARIOUS DOSES OF PGE_1

Expt. No.	Time Sacrificed	Dose	Plasma FFA	No. of Animals	*P* vs. Controls
	min	*μg/kg*	*μeq/liter*		
1	7	0	783 ± 18*	5	
		16	706 ± 96	5	N.S.
		80	532 ± 57	5	<0.01
		400	549 ± 45	5	<0.01
		1000	553 ± 38	5	<0.01
2	7	0	620 ± 60	5	
		16	513 ± 52	5	N.S.
		80	466 ± 50	5	<0.05
		400	463 ± 59	5	<0.05
3 and 4	15	0	520 ± 52*	10†	
		8	604 ± 52	10	N.S.
		80	820 ± 52	10	<0.001
		400	639 ± 52	10	≅0.13

All rats were fasted 19–20 hr prior to injection of compound. Several weeks elapsed between all experiments except between 3 and 4.

* Mean ± SEM.

† Five rats in experiment; the indicated standard errors are for five rats within an experiment and are based on the pooled variation arising in a completely randomized block design.

TABLE 2 EFFECT OF 80 μG/KG OF PGE_1 ON PLASMA FFA CONCENTRATION OF FASTED RATS 15 AND 30 MIN AFTER INJECTION

Time Sacrificed	Treatment	Plasma FFA	*P*
min		*μeq/liter*	
15	Saline	543 ± 28*	
	PGE_1	818 ± 28	<0.001
30	Saline	530 ± 28	
	PGE_1	565 ± 28	N.S.

All rats were fasted 19–20 hr prior to injection of PGE_1.

* Mean ± SEM, the latter based on pooled variation within the four groups (five animals per group).

TABLE 3 EFFECT OF PGE_1 ON FFA CONCENTRATION IN NONINCUBATED ADIPOSE TISSUE TAKEN FROM PRETREATED RATS

Treatment	Adipose Tissue FFA	*P*
	μeq/g	
Saline	7.2 ± 0.38*	—
80 μg of PGE_1 per kg	5.2 ± 0.37	<0.005

Animals were anesthetized with ether 7 min after injection of PGE_1 and epididymal adipose tissue was homogenized in Krebs-Ringer phosphate buffer as described in the text.

* Mean ± SEM. 10 animals per group, each fasted for 20 hr.

This demonstration of antilipolytic activity of PGE_1 in adipose tissue of rats fasted for 20 hr (Tables 1 and 3), in view of conflicting results of others (15, 16), raised the possibility that PGE_1 becomes ineffective when fasting is extended to 40 hr.

Consequently, the influence of PGE_1 on plasma and adipose tissue glycerol and FFA concentrations was determined in rats fasted for 40 hr. The antilipolytic effect of PGE_1 was again demonstrated (Table 4) since glycerol and FFA release from adipose tissue was inhibited and there was a lowering of glycerol and FFA concentrations in plasma. Furthermore, experiments 2, 2a, and 4 demonstrate that lipolysis in vivo and in vitro was equally inhibited in decapitated and anesthetized animals. Therefore, the possibility that ether anesthesia, unlike decapitation, may evoke sympathetic discharge and produce the observed results was eliminated.

The time course of activity of PGE_1 in fed rats was also studied. Table 5 shows that the plasma FFA concentration was decreased in fed rats after 7 min but that after 15 min and 30 min it was normal.

DISCUSSION

The studies described in this report demonstrate the antilipolytic effect of PGE_1 in vitro and in vivo, in fasted as well as fed rats. Interestingly, the nutritional state of the animal influences the course of events following i.v.

TABLE 4 INHIBITION OF LIPOLYSIS BY PGE_1 IN RATS FASTED FOR 40 HR

Expt. No.	Source	Glycerol	FFA
		μmoles/g	*μeq/g*
1	Nonincubated tissue	0.9 ± 0.2	3.2 ± 0.1
	Incubated 120 min (control)	4.8 ± 0.4	7.9 ± 0.7
	" + PGE_1 (1.0 μg/ml)	2.4 ± 0.2*	1.6 ± 0.1*
2	Nonincubated tissue	1.1 ± 0.2	5.1 ± 0.5
	Incubated 120 min (control)	4.7 ± 0.6	8.3 ± 1.3
	" + PGE_1 (1.0 μg/ml)	2.3 ± 0.2†	3.8 ± 0.4†
	" + PGE_1 (0.1 μg/ml)	2.5 ± 0.3†	4.3 ± 0.6†
2a	Nonincubated tissue	0.88 ± 0.1	4.4 ± 0.6
	Incubated 120 min (control)	4.1 ± 0.6	8.0 ± 1.0
	" + PGE_1 (1.0 μg/ml)	2.4 ± 0.1‡	4.6 ± 0.7‡
	" + PGE_1 (0.1 μg/ml)	2.2 ± 0.2‡	4.3 ± 0.6‡
	Plasma		
		μmoles/liter	*μeq/liter*
3	Control	260 ± 27	798 ± 26
	PGE_1 (80 μg/kg)	159 ± 16‡	576 ± 41*
4	Control	320 ± 14	—
	PGE_1 (80 μg/kg)	216 ± 11*	—

Adipose tissue, taken from animals fasted for 40 hr, was incubated in Krebs Ringer bicarbonate medium as described in the text. Animals in expts. 1, 2 and 3 were anesthetized with ether; in expts. 2a and 4 they were decapitated. Plasma was taken from animals 7 min after injection of PGE_1. Each number is the mean of duplicate determinations in six animals ± SEM.

* P vs. controls <0.001.

† P vs. controls <0.005.

‡ P vs. controls <0.01.

Glycerol and FFA release of incubated tissue in experiment 2a are not significantly different from those in experiment 2.

TABLE 5 PLASMA FFA CONCENTRATION IN RATS FED AD LIBITUM AFTER I.V. INJECTION OF PGE_1

Time	Dose	Plasma FFA		P
		Control	Treated	
min	*μg/kg*	*μeq/liter*		
7	80	266 ± 24*	197 ± 9*	0.01
	400	266 ± 24	185 ± 7	<0.01
15 (Expt. 1)	80	255 ± 10	258 ± 23	N.S.
15 (Expt. 2)	80	336 ± 13	340 ± 32	N.S.
30	80	293 ± 17	271 ± 7	N.S.

* Mean ± SEM. Five animals per group.

administration of the compound. For example, in fed rats, lowering of plasma FFA concentration, evident at 7 min, is followed by normal values at 15 min. In contrast, fasted rats show a biphasic response. After i.v. administration of PGE_1 plasma FFA concentrations are lowered at 7 min, elevated at 15 min, and normal at 30 min. These results agree qualitatively with those of Steinberg and Pittman (13) and of Bergström, Carlson, and Orö (12). Steinberg and Pittman showed that a single intravenous dose of PGE_1 decreases plasma FFA in the fasted dog. Since the effect persists for 1–2 hr, it is possible that the dog metabolizes or otherwise eliminates PGE_1 more slowly than the rat. Bergström and his coworkers reported that infusion of 0.2 μg of PGE_1 per kg per min for 30 min elevates plasma FFA concentration in the fed or fasted dog but that 0.4, 0.8, and 1.6 μg/kg per min decreases FFA concentration. Furthermore, they showed that the increase is abolished when a sympathetic ganglionic blocking agent is given. The data presented here agree with the latter finding in dogs if one assumes that immediately after injection the concentration of PGE_1 is high enough to inhibit lipolysis in adipose tissue, but in less than 15 min the concentration decreases enough for the inhibition to be overcome by the sympathetic response.

It is not possible to determine from these results why plasma FFA in the fed rat do not rebound above normal concentrations after 15 min. Perhaps PGE_1 is metabolized at different rates in fasted and fed rats and so the rebound occurs at another point in time. The difference could also be due to lower levels of activated lipase in the fed state.

Finally, one can only speculate on why other workers failed to demonstrate an effect of PGE_1 in fasted rats. The length of the fasting period does not appear to be a factor since in the present study PGE_1 was effective in rats fasted either for 20 or for 40 hr. The difference in timing in the in vivo experiments could account for the different results. Berti et al. (16) infused a dose of 140 μg/kg during 25 min and sacrificed the animals immediately. It is conceivable that at the end of the infusion the compensatory response already elevated plasma FFA from a previously depressed level. The procedure for studies in vitro in the present report differs from that of Bergström and Carlson (14) only in the source of the albumin (bovine vs. human). However, human albumin was found to give the same result (unpublished experiments). Stock and Westermann (15) used an albumin-free buffer without glucose. It is also possible that the strain of rat used in this work differs from other strains in its response to PGE_1.

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