Effects of infusion of some prostaglandins in essential fatty acid-deficient and normal rats

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ABSTRACT Infusion of 1 mg/kg per day of prostaglandin E_1 (PGE_1) for 2 and 7 wk failed to correct the dermal signs of essential fatty acid (EFA) deficiency in rats despite the known conversion of EFA to certain prostaglandins. PGE_1 caused no significant changes in serum cholesterol, triglycerides, or phospholipids or in liver neutral lipids in EFA-deficient or normal rats. In normal rats epinephrine-induced lipolysis was greater in fat pads from infused than from untreated rats. The effect on epinephrine-induced lipolysis was greater after the 7 wk infusion than after the 2 wk infusion. The 7 wk infusion also lowered plasma free fatty acid (FFA) concentrations. Infusion of PGE_2 and $PGF_{2\alpha}$ in combination for 4 wk had no significant effect on either dermal signs of EFA deficiency, lipolysis, or plasma FFA concentrations.

KEY WORDS prostaglandin E_1 · E_2 · $F_{2\alpha}$ · essential fatty acid deficiency · lipolysis · plasma free fatty acids

THE ESSENTIAL FATTY ACIDS, linoleic and arachidonic, completely reverse the signs of EFA deficiency in rats (1). Earlier work had shown that linoleic acid is converted to arachidonic acid in vivo (2, 3). The demonstrated conversion of certain all-*cis* C_{20} polyunsaturated acids to prostaglandins, for example arachidonic acid to PGE_2 (4, 5) and dihomo-γ-linolenic acid to PGE_1 (6) and $PGF_{1\alpha}$ (7), suggested the possibility of correcting signs of EFA deficiency with one of the prostaglandins. This communication reports results of studies on the effect of prolonged infusion of PGE_1, PGE_2, and $PGF_{2\alpha}$ in EFA-deficient and normal rats. While these studies were in progress, Van Dorp and coworkers reported preliminary studies on administration of PGE_1 to EFA-deficient rats and mice (8). A preliminary report of our work has been presented (9).

MATERIALS AND METHODS

Male weanling rats of Wistar origin were fed a fat-free diet (modified slightly according to Mohrhauer and Holman [1], personal communication) for about 3 months. Signs of EFA deficiency were obvious (scaly skin on feet and tail, stunted growth). Normal control rats were maintained on Purina laboratory chow. 1 wk before infusions were started, chronic indwelling venous cannulas were inserted under ether anesthesia (10). During the infusion period rats were in individual cages and wore a light-weight saddle connected to a cannula feed-through swivel (No. 1601, Lehigh Valley Electronics Engineering & Mfg. Co., Fogelsville, Pa.), which permitted relatively unrestrained movement (11).

Prostaglandins were given by continuous intravenous infusion, in a volume of 2.1 ml/day, by means of a syringe driver (No. 1100, Harvard Apparatus Co., Inc., Dover, Mass.). Stock solutions of 10 mg of prostaglandin per ml of ethanol were first diluted to 1 mg/ml with water and sufficient 0.01 N NaOH to make the pH 6, and then further diluted with 0.9% sodium chloride. All doses were based upon the average weight of the group of rats. In the first experiment PGE_1 was infused for 7 wk. Terminal studies (plasma FFA and lipolysis in adipose tissue) were made on rats in the fed state. Cholesterol (12), triglycerides (13), and phospholipids (14) were determined on blood samples drawn from the jugular vein of rats fasted overnight at 2, 4, and 6 wk. Three other experiments were carried out after 2 wk infusions of PGE_1, two with terminal measurements on fed rats, the other on rats fasted over-

Abbreviations: PGE_1, PGE_2, and $PGF_{2\alpha}$ are prostaglandin E_1, E_2, and $F_{2\alpha}$, respectively; EFA, essential fatty acid(s); FFA, free fatty acid(s).

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night. PGE_2 and $PGF_{2\alpha}$ were given as a mixture of 0.5 mg/kg per day of each for 4 wk. After infusions were stopped, rats were anesthetized with ether, a blood sample was drawn for determination of plasma FFA (15), and an epididymal fat pad was removed for measurement of basal and epinephrine-stimulated lipolysis (concentration of epinephrine, 0.1 μg/ml) (15). Liver lipids were extracted by the method of Folch, Lees, and Sloane Stanley (16). All terminal measurements were made about 1 hr after the infusion was stopped. Untreated rats received infusions of the saline vehicle.

RESULTS

Preliminary experiments indicated that rats would tolerate 1 mg/kg per day of PGE_1 without apparent ill effects, but 3.2 mg/kg per day caused severe swelling of the hind limbs after about 3 days. Accordingly, all doses were 1 mg/kg per day. The prostaglandins failed to correct the dermal signs of EFA deficiency in all experiments. The small groups of animals and wide individual variations made demonstration of small changes in other parameters impossible. Table 1 summarizes effects of the 7 wk infusion on lipolysis. Average plasma FFA concentrations were lower in PGE_1-treated than in untreated normal rats. Baseline lipolysis in fat pads was higher in EFA-deficient rats than normal rats, but PGE_1 infusion did not significantly change the rate. However, in the normal rats, epinephrine-induced lipolysis was significantly greater ($P < 0.01$) in fat pads from PGE_1-infused than in those from untreated rats. Too few of the EFA-deficient rats survived for a meaningful comparison to be possible.

Table 2 shows that PGE_1 infusion caused no significant changes in serum cholesterol, triglycerides, and phospholipids. Liver neutral lipids were higher in EFA-deficient rats, but were not affected by PGE_1.

The effects of 2-wk infusions of PGE_1 are summarized in Table 3. The basal lipolytic rate in the fat pads was higher in fasted rats than in fed rats. PGE_1 infusion had no significant effect on the magnitude

TABLE 1 EFFECT OF INFUSION OF PGE_1 FOR 7 WK ON PLASMA FFA AND LIPOLYSIS RATE IN ISOLATED RAT FAT PAD

Diet	Treatment	Number of Animals*	Plasma FFA	Lipolysis (FFA) Baseline	Lipolysis (FFA) Epinephrine-Stimulated
			μeq/liter	*μeq/g*	
Normal	Vehicle	6	418	1.13	10.17
	PGE_1	6	330†	1.13	22.33‡
	SD between samples within rats		11.2	0.38	2.60
	SD between rats		60.1	0.49	3.74
EFA-deficient	Vehicle	5	516	1.66	24.50
	PGE_1	3	323	2.22	25.93
	SD between samples within rats		26.7	0.43	3.93
	SD between rats		157.4	0.33	2.21

* Six rats started in each group. In the EFA-deficient groups, four rats (one vehicle, three PGE_1) died of unknown causes before the end of the experiment.
† P vs. vehicle <0.05.
‡ P vs. vehicle <0.01.

TABLE 2 EFFECT OF INFUSION OF PGE_1 FOR 6 WK ON SERUM LIPIDS OF NORMAL AND ESSENTIAL FATTY ACID-DEFICIENT RATS

Treatment	Number of Animals*	Cholesterol	Triglycerides	Phospholipids
		mg/100 ml		
Normal, untreated	6	92.3 ± 4.0†	73.7 ± 4.8†	129.0 ± 6.3†
Normal, PGE_1	6	100.3 ± 3.8	68.0 ± 8.2	129.5 ± 6.1
EFA-deficient, untreated	5	92.8 ± 13.4	54.6 ± 5.3	172.2 ± 33.5
EFA-deficient, PGE_1	4	82.0 ± 10.0	45.8 ± 12.3	130.0 ± 22.5

Blood samples were drawn from the jugular vein of rats after they were fasted overnight.
* Six rats started in each group. In the EFA-deficient groups, three rats (one untreated, two PGE_1) died of unknown causes before the end of the experiment.
† Mean ± SEM. P vs. untreated controls >0.1 in every case.

TABLE 3 EFFECT OF 2-WK INFUSIONS OF PGE_1 ON PLASMA FFA AND LIPOLYSIS RATE IN ISOLATED RAT FAT PAD

Diet and Nutritional State	Treatment	Number of Animals	Lipolysis (FFA) Baseline	Lipolysis (FFA) Epinephrine-Stimulated
			$\mu eq/g$	
Fasted, normal	Vehicle	5	2.12	14.65
	PGE_1	7	3.06	18.07
	SD between samples within rats		0.57	2.40
	SD between rats		0.64	3.74
Fasted, EFA-deficient	Vehicle	8	7.87	29.73
	PGE_1	5	8.57	23.95
	SD between samples within rats		2.05	4.76
	SD between rats		3.06	6.28
Fed, normal (expt. 1)	Vehicle	5	1.02	12.80
	PGE_1	5	1.22	16.73*
	SD between samples within rats		0.25	1.81
	SD between rats		0.31	2.39
Fed, normal (expt. 2)	Vehicle	6	0.72	18.23
	PGE_1	6	0.97	19.75†
	SD between samples within rats		0.44	2.79
	SD between rats		0.30	3.06

Experiment 1 with fed rats was terminated on February 28; experiment 2, on May 25.
* P vs. vehicle-infused controls <0.05.
† P vs. vehicle-infused controls >0.05. Data from the two experiments are analyzed as a 2 × 2 factorial, which showed significant differences for epinephrine-stimulated lipolysis between winter and spring rats ($P<0.01$) and also PGE_1-treated and untreated rats ($P<0.05$). Baseline lipolysis was unaffected.

of epinephrine stimulation. Two separate experiments were carried out on fat pads from fed, normal rats. In both, epinephrine stimulation was slightly greater in the PGE_1-infused groups. Data were analyzed as a 2 × 2 factorial (two treatments, two experiments), which showed significant differences for epinephrine-stimulated lipolysis between winter (expt. 1) and spring (expt. 2) rats ($P<0.01$) and also between PGE_1-treated and untreated rats ($P<0.05$). Baseline lipolysis was unaffected.

Infusion of the PGE_2 and $PGF_{2\alpha}$ mixture for 4 wk had no significant effect on either plasma FFA, baseline lipolysis, or epinephrine-stimulated lipolysis in the epididymal fat pad of either normal or EFA-deficient rats.

Prostaglandins are rapidly metabolized or removed from the blood by the lungs (17) and so might not have reached the tissues in effective concentrations. Accordingly, in another group of rats the infusion cannula was placed in the abdominal aorta just above the iliac bifurcation (cannula was modified from Weeks and Jones [18]). A 2 wk infusion of PGE_1 at 0.05 mg/kg per day did not affect dermal signs of deficiency. Infusion rates of 0.10 mg/kg per day and greater caused the hind limbs to swell.

DISCUSSION

The hypothesis that dermal signs of EFA deficiency might be due to secondary deficiency of prostaglandins was not substantiated. Gottenbos, Beerthuis, and Van Dorp (19) obtained similar negative results with PGE_1 in both EFA-deficient rats and mice treated both by oral administration and intravenous infusion for up to 2 wk. They also found no effect on the excessive transpiration of water through the skin of EFA-deficient animals.

The effect of the nutritional state on plasma FFA and lipolytic rate in the fat pad of normal and EFA-deficient animals agrees with the findings of Bergström and Carlson (20, 21). The inhibition by PGE_1 of baseline and hormonally-induced lipolysis in rat and human adipose tissue (22–24) as well as the inhibition of the fat-mobilizing effect of epinephrine in dogs (25–28) qualitatively resembles that of nicotinic acid. However, these studies show that, unlike nicotinic acid, PGE_1 does not lower serum cholesterol, triglycerides, or phospholipids. The greater lipolysis following epinephrine stimulation in spring compared to winter rats may have been a seasonal change, since fat metabolism (as indicated by a lower respiratory quotient) is more active in spring than winter rats (29).

Since the depression of plasma FFA following intravenous injection of PGE_1 in rats is reversed in 15 min (15), FFA may have been depressed during the infusion period in these experiments, but became normal before the animals were sacrificed. This possibility is reinforced by the finding that epinephrine-stimulated lipolysis is increased in normal rats by infusion of PGE_1. This increase in epinephrine-stimulated lipolysis is similar to that observed by Bizzi, Codegoni, Lietti, and Garattins (30) when 5-methylpyrazole-3-carboxylic acid, an inhibitor of lipid mobilization, was given to rats.

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