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Depression of Plasma FFA Levels in Unanesthetized Dogs by Single Intravenous Doses of Prostaglandin E_1. (31439)

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Prostaglandin, an acidic lipid fraction with vasodepressor and smooth muscle-stimulating properties, was first reported by Goldblatt(1, 2) and by von Euler(3,4). In recent years studies by Bergström and collaborators have led to the isolation and full characterization of a large family of structurally related prostaglandins(5-7). Members of the family have been shown to decrease basal rates of glycerol release and to inhibit the fat-mobilizing effects of lipolytic hormones in rat and human adipose tissue *in vitro*(8-10). Prostaglandin E_1 (PGE_1) was shown to block the *in vivo* fat-mobilizing effect of epinephrine in the anesthetized dog with little or no inhibition of its glucose-mobilizing effect(9,11). Bergström *et al* have shown in unanesthetized man an unexpected *rise* in plasma FFA during continuous infusion of small doses of PGE_1 alone and only a very limited inhibition of the fat-mobilizing action of simultaneously infused epinephrine(12).

Work reported here shows that intravenously administered PGE_1 counteracted the lipid-mobilizing effect of intravenous epinephrine in unanesthetized dogs. Glucose responses were little affected. Injection of large single doses of PGE_1 alone consistently produced a pronounced drop in basal plasma FFA levels which could not be explained as a secondary effect of the accompanying vasodepression.

Methods. Mongrel male dogs fasted overnight were trained to stand for several hours supported by a cloth sling. A venous catheter was placed in each foreleg: one for test substance injection, the other for blood sampling. Blood samples were heparinized and immediately chilled. Analyses were begun no more than 3 hours after sampling. Plasma FFA analyses were done according to the method of Dole(13) except that isooctane was used in place of heptane. Glucose determinations were performed enzymatically using glucose oxidase ("Glucostat" reagent sets, Worthington Biochemical Corp., Freehold, N. J.). PGE_1, kindly provided by Dr. Sune Bergström, and PGE_1-217 (9-keto-15-hydroxy-prosta-10,13-dienoic acid), a gift of Dr. J. E. Pike, Upjohn Co., were prepared in aqueous solutions containing 0.1 ml of ethanol for each mg of PGE_1 and diluted with 0.85% NaCl as necessary. The total amount of ethanol injected was never more than 0.1 ml. Stock epinephrine solutions (1 mg base/ml) were prepared from the bitartrate and diluted in 0.85% NaCl for use.

Hemodynamic measurements were made on trained conscious dogs. Externalized carotid artery-jugular vein shunts had been placed in these dogs at least 2 days prior to study. Blood pressure measurements were made from the arterial side of the shunt using a Statham strain gauge and Sanborn recorder system. Injections and sample withdrawals were made

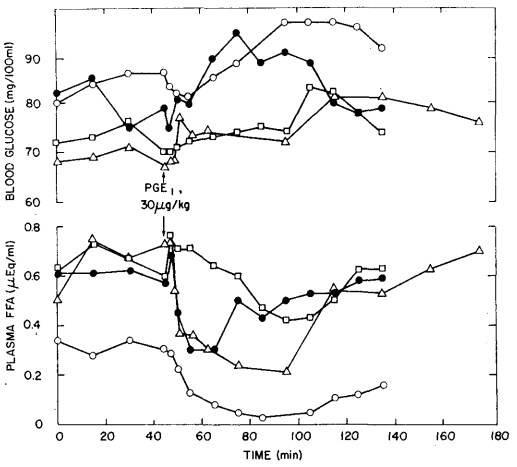

FIG. 1. Response of 4 conscious dogs before and after single injections of PGE_1.

through a catheter inserted in the venous side so that the tip rested in the right atrium or in the superior vena cava.

Results. Rapid single injections of 30 μg/kg of PGE_1 produced pronounced drops in plasma FFA levels (Fig. 1). Although the drop in each case began almost immediately after injection, rates of fall and degrees of depression differed from dog to dog. The blood glucose level in each case rose slightly after PGE_1 injection, but the changes were small and variable.

The possibility had to be considered that the profound fall in blood pressure known to occur with the doses of PGE_1 used in this study(9,10), although of short duration, might in itself contribute to the observed fall in FFA levels. For this reason the effects of intravenous PGE_1-217 and of nitroglycerine were tested. PGE_1-217 has been shown to possess vasodepressor potency equal to or greater than that of PGE_1 while exhibiting much weaker effects on lipid mobilization (14). As shown in Fig. 2 the vascular dynamic changes induced by PGE_1-217 were not accompanied by a drop in plasma FFA as occurred with injection of PGE_1 in the same dog. In fact there was a small but consistent rise in plasma FFA following injection of PGE_1-217. Injection of another vasodepressor, nitroglycerin, yielded similar results, *i.e.*, a slight rise in FFA level (Fig. 3). Subsequent injection of PGE_1 in the same experiment caused the expected fall in plasma FFA.

Fig. 4 illustrates a typical experiment in which PGE_1, injected with epinephrine, com-

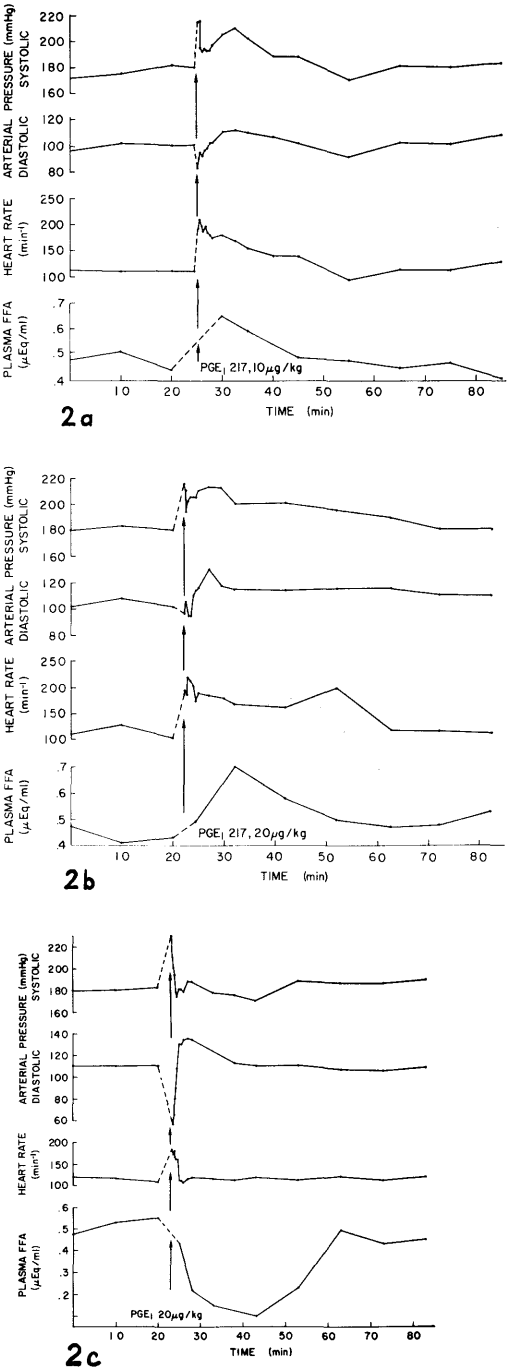

FIG. 2. Response of a conscious dog to 2 doses of PGE_1-217 and one dose of PGE_1.

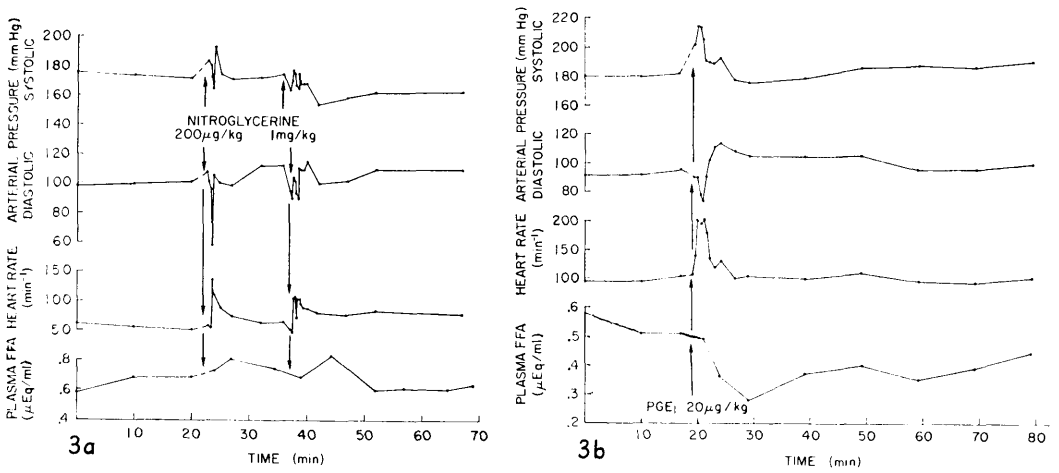

FIG. 3. Response of a conscious dog to 2 doses of nitroglycerin and one dose of PGE_1.

pletely blocked the usual epinephrine-induced plasma FFA rise. Indeed the FFA level was reduced far below control levels, where it remained for almost an hour. The epinephrine-induced rise in blood glucose was unaffected. Table I summarizes results of 3 additional experiments of this type. Absolute changes in plasma FFA and blood glucose levels are shown following rapid injections of PGE_1 alone, epinephrine alone, and finally PGE_1 with epinephrine. All dogs showed initial control plasma FFA values of between 0.6 and 1.1 μeq/ml. The first injection, PGE_1 alone in each case, depressed the plasma FFA levels to between 0.1 and 0.4 μeq/ml. The subsequent responses were over this low baseline level to which the FFA concentrations returned in each case. Initial blood glucose levels were between 75 and 85 mg/100 ml and values returned toward this more or less unchanged baseline after each epinephrine injection whether PGE_1 was given simultaneously or not.

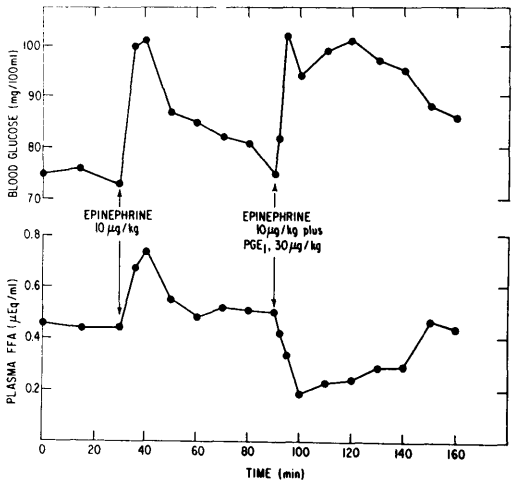

FIG. 4. Response of a conscious dog to an injection of epinephrine followed by an injection of epinephrine and PGE_1.

Discussion. It is shown that PGE_1, given in large single doses, depresses basal FFA levels and blocks epinephrine-induced elevation of FFA levels in intact conscious dogs. The results are compatible with a direct action of PGE_1 on adipose tissue analogous to that previously demonstrated action *in vitro* (8-10). The hyperglycemic response to epinephrine was apparently not affected, suggesting that PGE_1 inhibits the activation of adipose tissue lipase but not the activation of liver phosphorylase.

PGE_1-217, which has vasodepressor activity equal to or greater than that of PGE_1, caused a slight *rise* in plasma FFA levels, as did nitroglycerin. After both of these drugs, as after PGE_1, there was a marked tachycardia indicating a sympathetic discharge as expected in response to a vasodepressor substance. In the case of PGE_1 given in large doses the local inhibitory effect in the adipose tissue may predominate; in the case of PGE_1-217, which is much less potent than PGE_1 in inhibiting the lipolytic action of catecholamines (14), the sympathetic discharge is largely unopposed and hence plasma FFA

levels rise. This hypothesis may well account for the apparently paradoxical *rise* in FFA levels observed by Bergström *et al*(12) in that the dosage rates were sufficient to activate compensatory sympathetic discharge in an attempt to maintain blood pressure but insufficient to block the lipolytic effect of released catecholamines at the adipose tissue level. That there is a sympathetic discharge after PGE_1 administration is shown by the studies of Berti *et al*(15). They observed a marked hyperglycemia in rats given infusion of PGE_1 (5.6 μg/kg/min) but this was abolished by prior removal of the adrenal medulla. Recent studies by Carlson, Bergström and Orö have shown that while low rates of infusion of PGE_1 elevate FFA levels in dogs, higher rates cause a depression of FFA levels (16), findings they interpret along similar lines.

The persistence of low plasma FFA levels for 20-40 minutes after single injections of PGE_1 in the present studies was unexpected. As previously shown, the duration of the vasodepressor action of similar doses of PGE_1 is very short, return to control levels being reached within 3 to 5 minutes(9,11). Furthermore, it has been shown that the rate of breakdown of the prostaglandins *in vivo* is very high, and very little prostaglandin radioactivity can be demonstrated within adipose tissue autoradiographically after injections of tritium-labeled prostaglandin (Dr. Bengt Samuelsson, personal communication). The rather prolonged response suggests the possibility that the effect observed may in part be an indirect one, or that it is mediated by degradation products of PGE_1.

Hemodynamic measurements made here make it seem unlikely that the observed fall in plasma FFA on injection of PGE_1 is simply secondary to vasodepression. At the PGE dose levels used there was a drop in diastolic pressure but little change in mean blood pressure and the effect was transient. FFA levels remained low long after blood pressure had returned to normal. Furthermore similar cardiovascular changes due to injection of PGE_1-217 or nitroglycerin yielded small *rises* in plasma FFA levels.

Summary. Intravenous injection of large

TABLE I. Effects of Rapid Injection of PGE_1, Epinephrine, and PGE_1 with Epinephrine on Plasma FFA and Blood Glucose in 3 Dogs.

	Dog 1			Dog 2			Dog 3		
	PGE_1 (30 μg/kg)	Epi. (10 μg/kg)	PGE_1 (30 μg/kg) Epi. (10 μg/kg)	PGE_1 (20 μg/kg)	Epi. (10 μg/kg)	PGE_1 (20 μg/kg) Epi. (10 μg/kg)	PGE_1 (12.5 μg/kg)	Epi. (5 μg/kg)	PGE_1 (12.5 μg/kg) Epi. (5 μg/kg)
Change in plasma FFA (μeq/ml)	— .80	+ .38	+ .26	— .79	+ .42	+ .28	— .79	+ .17	+ .09
Change in blood glucose (mg/100 ml)	−5	+35	+23	−5	+34	+35	−5	+38	+36

doses of PGE_1 into unanesthetized dogs caused marked decreases in plasma FFA levels lasting up to 40 minutes. On the other hand, vasodepression produced by injection of similar doses of PGE_1-217 or by injection of nitroglycerin produced no fall in FFA levels, but instead a slight rise. This is interpreted as a response to sympathetic discharge induced by vasodepressor agents that do not share the ability of PGE_1 to block the lipolytic action of epinephrine on adipose tissue. Simultaneous intravenous injection of PGE_1 and epinephrine diminished or abolished the lipid-mobilizing action of the latter, but glucose response was unaffected.

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Activation of Intravascular Coagulation by Collagen.* (31440)

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It has been suggested that the activation of Factor XII (Hageman Factor) triggers the so-called "waterfall sequence" of intrinsic blood coagulation(1,2). It has recently been found that Factor XII is almost selectively adsorbed from human plasma by collagen fibers(3) and that this factor is activated following its adsorption(4). The purpose of the present study was to evaluate the effectiveness of collagen in activating blood coagulation *in vitro* and *in vivo*, and to study further the mechanism by which this occurs. The effect of collagen and elastin on blood coagulation *in vitro* was compared with that of inorganic surface active agents. Eluates from collagen previously exposed to plasma were also injected into rabbits, and tested for their ability to produce stasis thrombi in veins.

Materials. Undenatured collagen and elastin were obtained from Calbiochem, Los Angeles, Calif. Kaolin was obtained from the Fisher Scientific Co., Fair Lawn, N. J., and dicalite from the Great Lakes Carbon Corp., Los Angeles, Calif. Plastic test tubes, made of clear polystyrene, were obtained from Falcon Plastics Co., Los Angeles, Calif. New Zealand white rabbits, weighing approximately 3 kg, were used for obtaining blood and for

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