Prostaglandins: The Inhibition of Hepatic Cholesterol Ester Synthesase in the Rat (34677)

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Prostaglandins are widely distributed in tissue. They are found in high concentration in seminal and menstrual fluid; in lower concentration in lung, pancreas, kidney, and brain (1). Steinberg (2) showed that prostaglandin E_1 (PGE_1) reduced the release of glycerol and free fatty acids during incubation of rat epididymal fat pad and counteracted the fat-mobilizing activities of epinephrine, norepinephrine, adrenocorticotropic hormone, glucagon, and thyroid-stimulating hormone. Our group noted the stimulating effect on cholesterol ester synthesase of a number of hormonal compounds such as testosterone, 17β-esteradiol, L-thyroxine, and glucagon (3). We observed, as described below, a striking inhibition of this enzyme system in the synthesis of cholesteryl palmitate, oleate, and linoleate from cholesterol-7α-^{3}H *in vitro* on the addition of PGE_1 or $PGF_{1\alpha}$ to the incubate.

Materials and Methods. Livers from male Sprague-Dawley rats, 150–200 g, were homogenized in 0.1 *M* potassium phosphate buffer, pH 7.4, containing 0.25 moles of sucrose/1000 ml of buffer. A microsomal enzyme fraction was prepared as described by Goodman *et al.* (4). One μmole of cholesterol-7α-^{3}H (5.8 μCi) dissolved in 0.1 ml of propylene glycol and 0.3 μmoles each of potassium oleate, palmitate, and linoleate were placed in a 10-ml Erlenmeyer flask. The appropriate quantities of prostaglandins were added in 0.05 ml of propylene glycol. The control incubations received the identical quantity of propylene glycol without prostaglandin. After the addition of 1 μmole of coenzyme A, 9 mg of defatted human serum albumin, 18 μmoles of ATP and 2.2 mg of microsomal protein (determined by Lowry reaction) dissolved in 0.1 *M* potassium phosphate buffer, pH 7.4, to each flask, the incubations were performed in a Dubnoff metabolic incubator at 37° for 2 hr with air as gas phase. The final total incubation volume was 3 ml in each flask. The incubations were terminated by freezing in a Dry Ice–acetone mixture (—60°) and stored at —20° for 2 days. After the addition of 12,000 dpm (200 μg) each of cholesteryl-4-^{14}C oleate, palmitate, and linoleate as carrier, the incubations were extracted with ethyl ether. Purification and separation of the individual cholesterol-^{3}H-^{14}C esters was done by column chromatography on alumina and thin-layer chromatography on silica gel G impregnated with $AgNO_3$ as described previously (3). A constant $^3H/^{14}C$ ratio of the isolated cholesterol ester was accepted as a criterion for its radiochemical purity. Based on the recovery of ^{14}C-tracer, the losses of ^{3}H-labeled cholesterol ester incurred during the extraction and chromatographic procedures could be calculated. The data listed in Table I are correspondingly corrected.

Results. At a PGE_1 concentration of 9.2×10^{-8} molar, cholesterol ester synthesis was inhibited an average of 45%. At the highest concentration (6.9×10^{-7} *M*) cholesterol ester synthesis was inhibited an average of 91%. Inhibition increased with increasing quantities of PGE_1 or $PGF_{1\alpha}$. Although PGE_1 and $PGF_{1\alpha}$ have different biological activity when perfused in isolated intestinal smooth muscle and uterine preparations (5), no apparent differences in their inhibitory effects were noted between these two compounds in the present experiments at the higher concentrations used. Both compounds inhibited the enzymatic esterification in a similar fashion. The degree of inhibition of the individual

TABLE I. Effect of Prostaglandins E_1 and $F_{1\alpha}$ on Cholesterol Esterification by a Liver Microsomal Preparation.

Conc of prostaglandin (M)	Cholesteryl palmitate (μmoles formed)	Inhibition (%)	Cholesteryl oleate (μmoles formed)	Inhibition (%)	Cholesteryl linoleate (μmoles formed)	Inhibition (%)
None (control)	2.86 ± 0.6[b]		3.16 ± 0.3		2.95 ± 0.4	
PGE_1[a]						
4.6×10^{-8}	2.10 ± 0.5	26.6	2.63 ± 0.5	16.8	2.47 ± 0.5	16.3
9.2×10^{-8}	1.43 ± 0.1	50.0	1.80 ± 0.3	43.0	1.72 ± 0.1	41.7
4.6×10^{-7}	0.37 ± 0.05	87.0	0.45 ± 0.02	85.7	0.39 ± 0.03	86.8
6.9×10^{-7}	0.21 ± 0.02	92.7	0.26 ± 0.06	91.8	0.15 ± 0.09	94.9
$PGF_{1\alpha}$[c]						
4.6×10^{-8}	2.16 ± 0.3	24.5	2.26 ± 0.6	28.5	2.54 ± 0.5	13.9
9.2×10^{-8}	1.36 ± 0.2	52.4	1.63 ± 0.3	48.4	1.69 ± 0.2	42.7
4.6×10^{-7}	0.38 ± 0.1	86.7	0.37 ± 0.08	88.3	0.35 ± 0.09	88.1
6.9×10^{-7}	0.19 ± 0.05	93.3	0.22 ± 0.03	93.0	0.21 ± 0.03	92.9

[a] Prostaglandin E_1, U-10, 136; Lot No. 8111-JEP-76A.

[b] Values represent the mean ± standard error of four experiments.

[c] Prostaglandin $F_{1\alpha}$, U-18, 714; Lot No. 9222-JHK-104B.

ester formation differed only at the smallest concentration (4.6×10^{-8} *M*) of prostaglandins. Preliminary work has shown that at even higher concentrations of PGF_1 or $PGF_{1\alpha}$ (5×10^{-6} to 1×10^{-5} *M*) in the incubation medium the cholesterol ester biosynthesis was completely inhibited.

Discussion. From the results it is evident that PGE_1 and $PGF_{1\alpha}$ show a potent inhibitory effect on the esterification of cholesterol with long-chain fatty acids by liver microsomes. The physiological role of the prostaglandins and in particular their effect on liver metabolism is still obscure. Their remarkable biological potency possibly expresses itself in hormonal or regulatory roles in a variety of biochemical systems. In previous studies, we found that in the rat, hormonal agents such as androgens, estrogens, L-thyroxine, and glucagon affect the synthesis *in vitro* of cholesterol esters by liver microsomes (3). In addition, cholesterol ester metabolism *in vivo* is controlled by hormones (6). If the specific inhibitory effect of prostaglandins on hepatic cholesterol esterification is one of their physiological roles, this action might be related to the mobilization, modification, and release of free and ester cholesterol from liver depots into plasma lipoprotein. Further experiments will determine if prostaglandins are effective on the regulation of plasma free cholesterol and cholesterol esters. Atherosclerotic lesions are more easily induced in animals showing increased plasma levels of lipids and cholesterol. Prostaglandins affecting the lipid and cholesterol levels in plasma might thus enter into the process of atherosclerosis indirectly.

Summary. The effects of the prostaglandins E_1 and $F_{1\alpha}$ on the *in vitro* biosynthesis of cholesteryl palmitate, oleate, and linoleate by rat liver microsomes from cholesterol and free fatty acids was studied. Both prostaglandins exerted an inhibitory action on the enzymatic esterification of cholesterol. The degree of inhibition increased with increasing concentrations of PGE_1 and $PGF_{1\alpha}$ in the incubation medium. At the lowest concentration used (4.6×10^{-8} *M*) cholesteryl linoleate synthesis showed the least inhibition (about 15%) while cholesteryl palmitate formation was inhibited by about 25%. At the higher concentrations of PGE_1 and $PGF_{1\alpha}$ used, no difference in the degree of inhibition of the individual esters was observed.

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