Effects of epinephrine on plasma cholesterol levels in rats

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Abstract The present study was undertaken to evaluate whether epinephrine increases plasma cholesterol in rats. Epinephrine suspended in sesame oil was subcutaneously administered at 21:00 hr (9 PM). Blood was drawn 12 hr later, and plasma cholesterol was shown to be increased by epinephrine in a dose-dependent manner (0.5–2.0 mg/kg). This epinephrine-induced hypercholesterolemia was enhanced by phentolamine (25 mg/kg) and inhibited by propranolol (25 mg/kg). Although the effect of epinephrine in normal rats was abolished by adrenalectomy, corticosterone (10 mg/kg) increased plasma cholesterol in both normal and adrenalectomized rats. These results demonstrate that epinephrine increases plasma cholesterol levels in rats, and that the effect of epinephrine appears to be mediated by the β-adrenergic receptors, depending upon adequate amounts of corticosteroids.—**Kunihara, M., and T. Oshima.** Effects of epinephrine on plasma cholesterol levels in rats. *J. Lipid Res.* 1983. **24:** 639–644.

Supplementary key words propranolol • phentolamine • corticosterone • adrenalectomy

Blood cholesterol is known to be a risk factor for atherosclerosis (1–3). It is, therefore, important to maintain appropriate constant levels of blood cholesterol. However, there are many reports of abnormal levels in hormonally imbalanced patients and experimental animals (4). In thyroid hormone-related conditions, blood cholesterol was reported to decrease in hyperthyroidism and increase in hypothyroidism (5–7). Blood cholesterol is also affected by adrenal cortex hormones; in Cushing's disease and continuous stress, blood cholesterol is elevated while in Addison's disease it is reduced (8–10). These reports suggest that hormones play important roles in the regulation of blood cholesterol metabolism.

Epinephrine is known to stimulate activities of rat hepatic 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, a rate-limiting enzyme in cholesterol biosynthesis (11–13). Kaplan, Jacques, and Gant (14) and Barrett (15) reported that blood cholesterol was elevated by an oil suspension of epinephrine or a constant infusion of epinephrine in dogs and rabbits. However, there are conflicting reports about the effects of epinephrine in rats. Shafrir and co-workers (16, 17) have shown that it elevates blood cholesterol, while Barrett (15) has reported that it does not. In the present study we examined whether epinephrine increases blood cholesterol in rats, and also studied the relationships between epinephrine and corticosterone in influencing blood cholesterol levels.

MATERIALS AND METHODS

Chemicals

Epinephrine, propranolol, and corticosterone were purchased from the Sigma Co. Phentolamine was from the Ciba-Geigy Pharmaceutical Co. All other reagents were of the highest grade commercially available. Chemicals in Wako kits (Wako Pure Industry Ltd., Tokyo) were available to determine plasma cholesterol, triglyceride, and phospholipids.

Animals

Male Sprague-Dawley rats weighing 200–270 g were housed two per cage. The temperature of the animal room was maintained at 23 ± 1°C and relative humidity at 55 ± 5%. The room was lighted from 07:00 hr to 19:00 hr. Standard laboratory chow and water were available ad libitum during the experiments. Adrenalectomy was performed under pentobarbital anesthesia (50 mg/kg). The adrenalectomized animals were given 0.9% NaCl solution in place of drinking water, and they were used 7 days after the operation.

Experimental designs

Epinephrine (0.5, 1.0, and 2.0 mg/kg), phentolamine (25 mg/kg), propranolol (25 mg/kg), and corticosterone (10 mg/kg) were used in the present study. The drugs were suspended in sesame oil and given subcutaneously to the animals at 21:00 hr. Blood was obtained

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from the tail vein at 0 hr and 12 hr later. Blood was also obtained after decapitation of the rats 12 hr after the administration of drugs in studies on the combined effects of epinephrine with a β- or an α-adrenergic blocker.

Analytical methods and calculations

Total and free cholesterol in plasma were assayed by an enzymatic method (18, 19). Cholesterol in the high density lipoprotein (HDL) fraction was determined by the modified method of Lopes-Virella et al. (20). A phosphotungstate-magnesium chloride solution was added to the plasma sample to precipitate chylomicron, very low density lipoprotein (VLDL), and low density lipoprotein (LDL) fractions. After centrifugation, cholesterol in the supernatant (HDL fraction) was assayed by the same enzymatic method. The ratio of the β fraction (chylomicron, VLDL, and LDL) to the α fraction (HDL), one of the atherosclerosis indexes, was calculated as follows: (total cholesterol − HDL cholesterol)/HDL cholesterol = β/α. Triglyceride, phospholipids, and free fatty acids in plasma were determined with commercial kits (21–24).

Student's *t*-test was employed to determine the statistical significance of the data.

RESULTS

Increases in plasma cholesterol levels by epinephrine

Preliminary experiments revealed that there were no changes in plasma cholesterol for at least 6 hr following the subcutaneous administration of epinephrine (0.2 mg/kg) in saline.

Sesame oil as a control produced no significant increase in plasma cholesterol 12 and 24 hr after its administration (**Fig. 1**). Epinephrine significantly increased plasma cholesterol in a dose-dependent manner 12 hr after administration. Doses of 0.5, 1.0, and 2.0 mg/kg of epinephrine resulted in increases of 38.1, 48.0 and 49.0% in plasma cholesterol, respectively. Plasma cholesterol was significantly elevated even 24 hr after epinephrine administration.

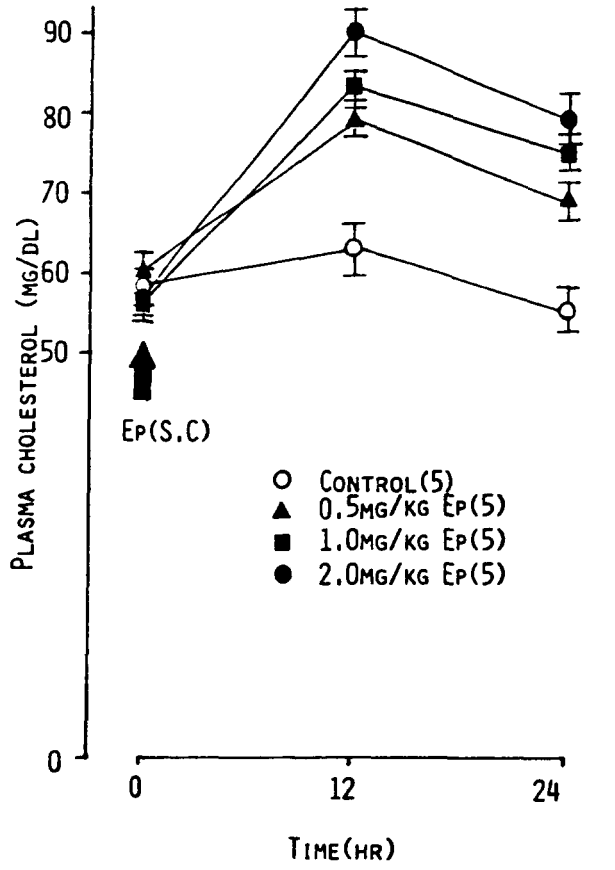

Fig. 1. Increases in plasma cholesterol levels produced by epinephrine. Epinephrine (0.5, 1.0, and 2.0 mg/kg) in sesame oil was administered subcutaneously at 21:00 hr. Number of animals shown in parentheses. Ep, epinephrine.

Effects of the α-adrenergic blocker on epinephrine-induced hypercholesterolemia

Fig. 2 shows the percent change in plasma cholesterol 12 hr after the administration of epinephrine in combination with phentolamine. Plasma cholesterol was significantly increased by a single administration of 1.0 mg/kg of epinephrine (32.5%) or 25 mg/kg of phentolamine (20.5%). Plasma cholesterol was more markedly increased (54.7%) when epinephrine was combined with phentolamine.

Table 1 shows plasma lipids 12 hr after drug administration. Total cholesterol was significantly elevated by epinephrine, phentolamine, and the simultaneous administration of these two drugs. Free cholesterol and the ratio of esterified cholesterol to total cholesterol also increased in all groups, however, these changes were not significant. HDL cholesterol was significantly elevated by epinephrine. The simultaneous administration of epinephrine and phentolamine showed significant elevations of HDL cholesterol and β/α values. Phospholipids were not affected in all groups. Triglyceride was decreased and free fatty acids were increased by epinephrine and the combination of the two drugs.

Effects of the β-adrenergic blocker on epinephrine-induced hypercholesterolemia

Fig. 3 shows the percent change in plasma cholesterol 12 hr after the administration of epinephrine in com-

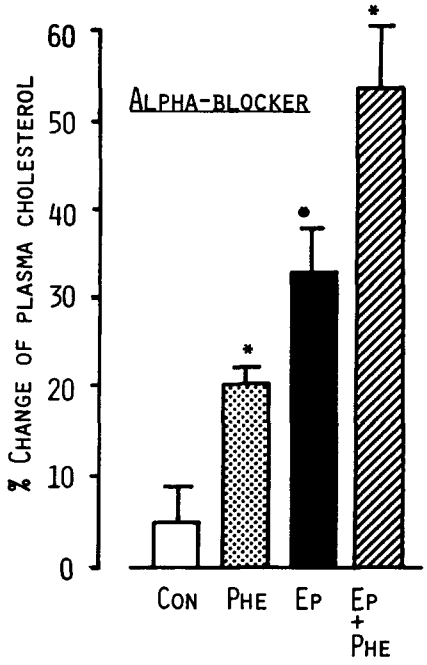

Fig. 2. Effects of the α-adrenergic blocker on epinephrine-induced hypercholesterolemia. Drugs were administered subcutaneously at 21:00 hr. Data are expressed as the percent increases in plasma cholesterol levels 12 hr later, compared to pre-administration levels. Each group consists of five animals. *, $P < 0.05$ compared to control. Con, control; Ep, epinephrine (1.0 mg/kg); Phe, phentolamine (25 mg/kg).

bination with propranolol. Epinephrine increased plasma cholesterol significantly (37.4%), but 25 mg/kg of propranolol did not (9.6%). The simultaneous administration of these two drugs showed no significant increase in plasma cholesterol (22.7%).

Table 2 shows plasma lipid levels 12 hr after drug administration. Total, free, and HDL cholesterol were significantly increased only by epinephrine. The relative amount of esterified cholesterol, β/α values, and phospholipids were not changed in all groups. Epinephrine also significantly decreased triglycerides and increased free fatty acids. The effects of epinephrine were blocked by propranolol.

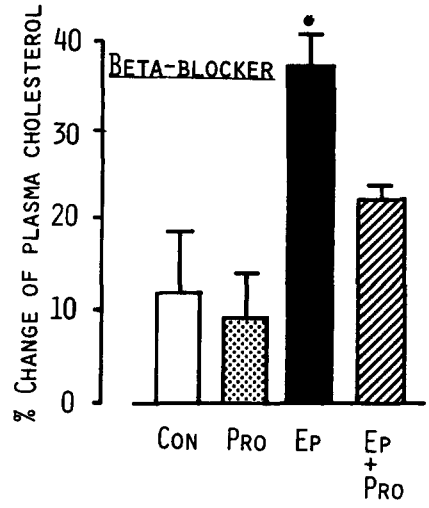

Fig. 3. Effects of the β-adrenergic blocker on epinephrine-induced hypercholesterolemia. Data are expressed as in Fig. 2. Each group consists of five animals. *, $P < 0.05$ compared to control. Con, control; Ep, epinephrine (1.0 mg/kg); Pro, propanolol (25 mg/kg).

Relationship between epinephrine and corticosterone in blood cholesterol levels

Fig. 4 shows the percent change in plasma cholesterol 12 hr after the administration of corticosterone in normal rats. Plasma cholesterol was significantly elevated by either 10 mg/kg of corticosterone (23.0%) or epinephrine alone (29.7%). The simultaneous administration of these two drugs also significantly increased plasma cholesterol (28.8%).

Plasma cholesterol was the same in normal and adrenalectomized rats, 60.8 and 65.7 mg/dl, respectively.

Fig. 5 shows the percent change in plasma cholesterol 12 hr after the drug administration in adrenalectomized rats. Plasma cholesterol was markedly increased by corticosterone (30.4%). In contrast, the increase in plasma cholesterol by epinephrine in normal rats was inhibited by adrenalectomy (7.9%). However, plasma cholesterol was significantly increased by simultaneous administration of these two drugs (37.5%).

TABLE 1. Changes on plasma lipids 12 hr after simultaneous administration of epinephrine and phentolamine

	T-Chol	F-Chol	HDL-Chol	E/T[a]	β/α	PL	TG	FFA
		mg/dl		%		*mg/dl*		*μEq/l*
Control	60.2 ± 1.5[b]	15.8 ± 0.9	30.8 ± 1.3	73.8 ± 1.3	0.97 ± 0.07	121 ± 2	85.5 ± 13.2	177 ± 19
Phe (25 mg/kg)	67.2 ± 1.8*	17.2 ± 1.4	30.8 ± 0.6	74.4 ± 1.7	1.19 ± 0.08	128 ± 6	85.0 ± 14.3	225 ± 31
Ep (1.0 mg/kg)	75.2 ± 5.0*	17.8 ± 1.2	36.9 ± 2.0*	76.2 ± 1.3	1.04 ± 0.08	129 ± 5	63.0 ± 4.8	253 ± 51
Phe + Ep	83.7 ± 5.3*	19.8 ± 1.5	36.6 ± 2.1*	76.4 ± 0.6	1.29 ± 0.07*	120 ± 8	31.0 ± 3.7*	365 ± 81*

Drugs were suspended in sesame oil and administered simultaneously at 21:00 hr. Animals were killed by decapitation 12 hr later. Total cholesterol (T-Chol), free cholesterol (F-Chol), HDL-cholesterol (HDL-Chol), phospholipids (PL), triglyceride (TG), and free fatty acids (FFA) in plasma were determined by enzymatic methods. Phe, phentolamine; Ep, epinephrine. *, $P < 0.05$ compared to the control.

[a] E/T shows the percentage of cholesterol in esterified form.

[b] Data are expressed as means ± SEM of five animals.

TABLE 2. Changes in plasma lipids 12 hr after simultaneous administration of epinephrine and propranolol[a]

	T-Chol	F-Chol	HDL-Chol	E/T	β/α	PL	TG	FFA
		mg/dl		%		*mg/dl*		*μEq/l*
Control	53.2 ± 2.2[b]	11.4 ± 0.9	26.0 ± 1.5	78.7 ± 1.0	1.06 ± 0.09	128 ± 4	114.7 ± 7.0	226 ± 30
Pro (25 mg/kg)	59.3 ± 5.0	12.4 ± 0.8	30.1 ± 1.0*	76.6 ± 2.0	1.12 ± 0.11	124 ± 4	64.1 ± 4.2*	263 ± 50
Ep (1.1 mg/kg)	64.8 ± 3.3*	15.6 ± 1.0*	34.4 ± 2.0*	75.7 ± 1.0	0.87 ± 0.10	119 ± 3	51.4 ± 5.9*	519 ± 46*
Pro + Ep	51.9 ± 2.4	13.6 ± 1.9	28.1 ± 2.2	76.0 ± 1.0	1.05 ± 0.11	121 ± 5	96.9 ± 6.3	221 ± 43

[a] See legend to Table 1 for details. Pro, propranolol; Ep, epinephrine. *, $P < 0.05$ compared to the control.
[b] Data are expressed as means ± SEM of five animals.

DISCUSSION

HMG-CoA reductase, a rate-limiting enzyme in cholesterol biosynthesis (25), has a circadian rhythm (26). It is stimulated by epinephrine during the dark period, but not during the light period (11, 12). When epinephrine was dissolved in 0.9% NaCl solution and subcutaneously administered (0.2 mg/kg), there were no changes in plasma cholesterol levels for at least 6 hr. In the present study, epinephrine was suspended in sesame oil to prolong its effects. When this suspension was administered during the dark period (at 21:00 hr), plasma cholesterol levels were elevated in a dose-dependent manner (Fig. 1). Barrett (15) reported that plasma cholesterol levels increased in dogs and rabbits but not in rats 24 hr after administration of epinephrine in oil. In contrast, Shafrir et al. (16, 17) showed that serum cholesterol levels were significantly elevated by epinephrine in oil suspension in rats. In this study, plasma cholesterol levels were elevated 20 to 30% 12 hr after a single administration of epinephrine oil suspension (Fig. 1). These results agree with Shafrir's observations. However, they administered epinephrine once a day for 2 days. Our results showed that a single administration is enough for the observation of the effects of epinephrine on blood cholesterol, and that the number of drug administrations and the period of blood collection employed by Barrett were not adequate.

In the present study, epinephrine-induced hypercholesterolemia was enhanced by phentolamine and inhibited by propranolol (Figs. 2 and 3), suggesting the participation of β-adrenergic mechanisms. It is well known

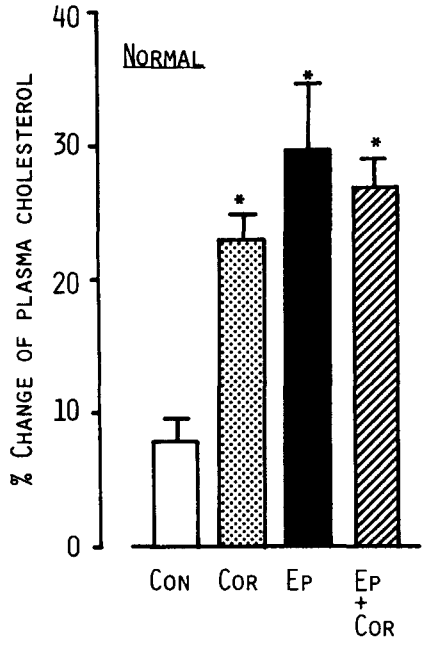

Fig. 4. Combined effects of epinephrine and corticosterone on plasma cholesterol levels in normal rats. Data are expressed as in Fig. 2. Each group consists of five animals. *, $P < 0.05$ compared to control. Con, control; Ep, epinephrine (1.0 mg/kg); Cor, corticosterone (10 mg/kg).

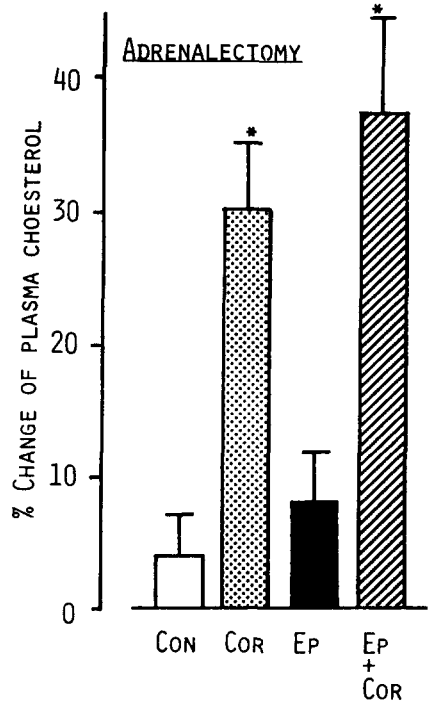

Fig. 5. Combined effects of epinephrine and corticosterone on plasma cholesterol levels in adrenalectomized rats. Data are expressed as in Figs. 2 and 4. Each group consists of five animals. *, $P < 0.05$ compared to control. Con, control; Ep, epinephrine (1.0 mg/kg); Cor, corticosterone (10 mg/kg).

that epinephrine elevates hepatic cyclic AMP via β-adrenergic receptors (27, 28). However, there are many reports that glucagon and dibutyryl cyclic AMP inhibited not only the secretion of cholesterol from the liver but also hepatic HMG-CoA reductase activity (29–31). Therefore, one can hardly conclude that the hypercholesterolemia induced by epinephrine is due to the elevation of hepatic cyclic AMP levels. Edwards (13) showed that serum augmented the effect of epinephrine on HMG CoA reductase activity in isolated rat hepatocytes. The present study also revealed that plasma free fatty acids changed in the same direction as plasma cholesterol, while plasma triglyceride changed in an opposite direction (Tables 1 and 2). Gho and Heimberg (32–34) reported that free fatty acids, especially, oleic acid, stimulated hepatic secretion and biosynthesis of cholesterol in the isolated perfused rat liver. Their reports suggest one possible explanation: the stimulation of lipolysis by epinephrine, presumably through the β-adrenergic mechanism, results in the elevation of blood fatty acids, which might stimulate the biosynthesis and secretion of hepatic cholesterol to elevate blood cholesterol levels.

In adrenalectomized rats, the effect of epinephrine was abolished, but reappeared after combined administration with corticosterone (Fig. 5). These results showed that the effect of epinephrine requires an adequate amount of corticosteroids, suggesting a permissive effect of adrenal cortex hormones. These results agree with those of Shafrir et al. (16, 17). The present study also revealed that corticosterone elevated plasma cholesterol levels in normal and even in adrenalectomized rats (Figs. 4 and 5). Recent reports have shown that the diurnal rhythms of activity of HMG-CoA reductase and cholesterol 7α-hydroxylase, rate-limiting enzymes of cholesterol biosynthesis and excretion, are stimulated by corticosterone in vitro (11, 35, 36). Therefore, our data suggest the possibility that corticosterone increased blood cholesterol directly by the stimulation of these two hepatic key enzymes as well as by a permissive effect.

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