

# Supersensitivity of Atherosclerotic Rabbit Aorta to Ergonovine

## MEDIATION BY A SEROTONERGIC MECHANISM

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**ABSTRACT** Patients with coronary vasospasm appear to be supersensitive to the coronary constrictor effects of ergonovine. To determine whether atherosclerosis alters arterial reactivity and sensitizes arteries to ergonovine, contractile responses of isolated aortae from control rabbits and from rabbits fed a high-cholesterol diet were compared. Aortic strips were mounted in a myograph for the monitoring of isometric tension, equilibrated in oxygenated Krebs buffer, and exposed to graded concentrations of agonists and antagonists. The concentration-response relation for ergonovine in atherosclerotic arteries exhibited a markedly depressed constrictor threshold concentration (0.5 pM vs. 0.23  $\mu$ M in controls), a significantly lowered one-half effective dose ( $ED_{50}$ ) value, and an augmented maximal response. Furthermore, atherosclerotic arteries showed similar, although less pronounced changes in the concentration-response relation for serotonin. In contrast, responses to 34 mM KCl were virtually identical, and the concentration-response relation for phenylephrine were similar in the two groups. In control arteries, 0.1  $\mu$ M phentolamine and 0.1  $\mu$ M prazosin suppressed responses to 1  $\mu$ M ergonovine by 71 and 90%, respectively. However, in atherosclerotic arteries  $\alpha$ -blockers in the same concentration inhibited responses to 0.01  $\mu$ M ergonovine by less than 10%. On the other hand, 0.1  $\mu$ M cyproheptadine, a serotonergic antagonist, suppressed these responses by 82%. Thus, the supersensitivity to ergonovine appeared to be mediated predominantly by a serotonergic mechanism. These results indicate that smooth muscle in atherosclerotic arteries may be supersensitive to specific vasoconstricting stimuli, a change that might contribute to arterial dysfunction in vivo.

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<sup>1</sup>Abbreviation used in this paper:  $ED_{50}$ , drug concentration producing a half-maximal response.

## INTRODUCTION

There is increasing evidence that inappropriate coronary vasomotion may play an important role in the pathophysiology of ischemic heart disease (1, 2). Clinical studies have demonstrated that spastic constriction of large epicardial coronary arteries may precipitate spontaneous (1, 2) and exercise-related (3-9) episodes of myocardial ischemia, and trigger life-threatening ventricular dysrhythmias (1-9).

Of considerable interest is the observation that individuals suffering from coronary vasospastic disease appear to be susceptible to the coronary constrictor effects of the ergot alkaloid ergonovine (ergometrine) (1-9). Ergonovine-induced coronary artery constrictions have been reported to closely resemble spontaneously occurring spasms (10), and the alkaloid is now used routinely as a provocative agent for the angiographic diagnosis of coronary vasospasm (11).

In this study, we have investigated whether arteries from animals with diet-induced atherosclerosis undergo changes in contractility that might help elucidate the pathogenic mechanism of coronary vasospasm in man. Results demonstrate that isolated aortae from rabbits fed a high-cholesterol diet are supersensitive to the constrictor effects of ergonovine. This finding is in agreement with previous studies (12, 13) indicating that changes in the lipid environment of vascular smooth muscle may alter arterial contractility.

## METHODS

New Zealand White rabbits of either sex initially weighing between 2.0 and 2.5 kg were assigned at random to two dietary groups. One group was maintained on standard rabbit pellets, and the other received 2% cholesterol pellets (ICN Nutritional Biochemicals, Cleveland, Ohio). All rabbits were kept on the diets for a period of 9-10 wk.

During the last week of the feeding period, control and cholesterol-fed rabbits were lightly anesthetized with pentobarbital (20-25 mg/kg, i.v.), and a skin incision performed on the right side of the neck. Polyvinyl catheters (i.d., 0.5 mm;

o.d., 0.9 mm) were implanted into the carotid artery and jugular vein. The free ends of the catheters were exteriorized posteriorly below the base of the skull and connected to plastic stopcocks. Two to three days later, the conscious rabbits were placed without restraint in small cages and allowed to settle down. The catheters were connected to Statham P23 Db pressure transducers (Statham Instruments, Inc., Oxnard, Calif.), which were placed at mid-chest level. Records of arterial and venous pressure were obtained using Brush model 11-4307-04 transducer amplifiers and a Brush model 220 recorder (Gould Inc., Instrument Systems Div., Cleveland, Ohio).

At the end of the cholesterol-feeding period, blood samples were collected into EDTA-Vacutainer tubes (Becton, Dickinson & Co., Rutherford, N. J.; final EDTA concentration, 1 mg/dl), and the rabbits were killed by a blow to the base of the skull. The descending thoracic aorta was promptly excised, cleaned of surrounding tissue, and cut into spiral strips 2 mm in width and 15 mm in length (14). The helical strips were mounted for the recording of isometric force in an organ bath containing 100 ml of standard buffer of the following formulation (mM): NaCl, 118; KCl, 4.0; CaCl<sub>2</sub>, 1.5; NaH<sub>2</sub>PO<sub>4</sub>, 1.2; MgSO<sub>4</sub>, 1.2; NaHCO<sub>3</sub>, 25; and dextrose, 5 (15). After equilibration with a 95% O<sub>2</sub>-5% CO<sub>2</sub> gas mixture, the pH of the buffer was 7.39. Isometric force was normalized for the cross-sectional area of the strip and expressed in milligrams per square millimeter. Cross-sectional area was estimated by dividing the postexperimental wet weight of the strip by its length, a calculation that assumes a tissue density of 1. An initial preload of 2 g was applied to the strips. The preloaded preparations underwent a stress-relaxation. During an equilibration period of 2 h, preload in strips from control and cholesterol-fed rabbits declined by  $0.451 \pm 0.032$  g (SE;  $n = 15$ ) and  $0.438 \pm 0.038$  g ( $n = 14$ ) ( $P > 0.1$ ), respectively. At the end of the equilibration period, preload was readjusted to exactly 1.5 g, and a test contraction was elicited by raising the KCl concentration of the buffer to 34 mM. The arteries were then relaxed by rinsing them with standard buffer.

Concentration-response relations for agonists were determined by the cumulative addition of drug to the bath fluid. Pilot experiments showed that repeated exposure of strips to ergonovine decreased their responsiveness to the drug. Because of this desensitization phenomenon (14), concentration-response experiments with ergonovine were not repeated in the same preparation, i.e., threshold concentrations were always determined in strips not previously exposed to a higher drug concentration.

To compare the effects of two agonists, the dose-response relation for each agent was determined sequentially in the same strip. The order of drug testing was alternated in successive experiments, and a rest period of 1 h was interposed between the two concentration-response experiments. Results obtained with the two orders of testing were very similar and were combined for analysis.

To compare the effects of antagonists, two different methods were used. In one procedure, matched strips cut from contiguous portions of an artery were equilibrated for 2 h with or without an inhibitor, and subsequently exposed to graded concentrations of an appropriate agonist. In the other procedure, arterial tone was first raised with an agonist, and the strip relaxed with a selected concentration of an antagonist. Arterial tone was allowed to fully equilibrate after addition of the antagonist. This method was applied only when the agonist produced stable, sustained increases in arterial tone. This was the case for ergonovine and phenylephrine. Force developed in response to 10 nM ( $n = 6$ ) or 10  $\mu$ M ( $n = 8$ ) ergonovine, and to 1  $\mu$ M phenylephrine ( $n = 8$ ) declined by

less than 3% during the first hour after addition of the drugs. On the other hand, this method was not applied when serotonin was the agonist, as this drug failed to produce stable force plateaus even when serotonin uptake was inhibited by the addition of up to 1  $\mu$ M fluoxetine (16).

At the end of the experiment the arteries were minced and homogenized in standard buffer in a Duall glass (Duall Plastics Div., Whittaker Corp., Athol, Mass.) glass homogenizer. Protein in the whole homogenate was estimated by the method of Lowry et al. (17) with serum bovine albumin as the standard. Total cholesterol in the same samples were measured enzymatically essentially as described by Gamble et al. (18). Total cholesterol in EDTA-plasma samples was measured by the same method.

All results were expressed as mean  $\pm$  SE. The significance of the difference between group means was assessed by the *t* test for unpaired samples. The difference between sequential mean values in the same group was assessed by the *t* test for paired samples.

Ergonovine maleate, L-phenylephrine, serotonin (5-hydroxytryptamine creatine sulfate), and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, Mo. Other drugs were gifts from chemical companies: phentolamine (Ciba-Geigy Corp., Summit, N. J.), prazosin (Pfizer, Inc., New York), cyproheptadine and indomethacin (Merck Sharp & Dohme Canada LTD, Montreal), L-propranolol (Ayerst Laboratories, Div., American Home Products Corp., New York), and fluoxetine (Eli Lilly and Co., Indianapolis, Ind.).

## RESULTS

*Effects of diet on hemodynamics.* Mean arterial pressure, mean venous pressure, and heart rate in conscious, unrestrained control rabbits ( $n = 18$ ) averaged  $82.8 \pm 3.1$  mm Hg,  $2.1 \pm 0.1$  mm Hg, and  $199 \pm 4$  beats/min. Corresponding values in conscious cholesterol-fed rabbits ( $n = 18$ ) did not differ significantly from the control values ( $P > 0.1$ ), and averaged  $84.0 \pm 3.9$  mm Hg,  $1.9 \pm 0.1$  mm Hg, and  $198 \pm 5$  beats/min.

*Effects of diet on plasma and arterial tissue cholesterol.* Total cholesterol in plasma from control and cholesterol-fed rabbits averaged at the end of the feeding period  $44 \pm 7$  and  $1978 \pm 178$  mg/dl ( $n = 18$ ), respectively. Total cholesterol concentration in the aortic strips was  $6.9 \pm 0.7$  mg/g wet wt or  $28.2 \pm 3.1$  mg/g aortic protein for the control group ( $n = 18$ ), and  $27 \pm 5$  mg/g wet wt or  $134 \pm 19$  mg/g aortic protein for the cholesterol-fed group ( $n = 18$ ). Values of aortic cholesterol are in agreement with those recently reported by Chan et al. (19).

*Effects of ergonovine, phenylephrine, and serotonin.* The log concentration-response relations for ergonovine in arteries from control and atherosclerotic arteries did not show a typical sigmoid shape and differed appreciably between the two groups (Fig. 1). Subnanomolar concentrations of ergonovine produced significant contractions in atherosclerotic strips, but had no effect on normal arteries. Threshold concentrations, one-half effective dose (ED<sub>50</sub>) values, and maximum responses derived from the dose-response

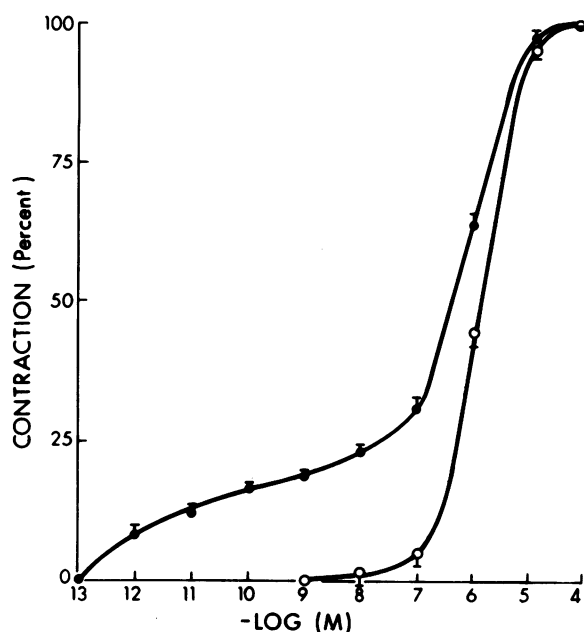


FIGURE 1 Concentration-response relations for ergonovine in aortic strips from control (○) and cholesterol-fed (●) rabbits ( $n = 9$ ). Vertical bars indicate 1 SE. Threshold concentrations,  $ED_{50}$ , and maximum responses derived from the same experiments are shown in Table I.

experiments are shown in Table I. The threshold concentration in atherosclerotic arteries was  $\sim 100,000$  times lower than that in the control arteries. In addition, the  $ED_{50}$  was significantly reduced and the maximum response significantly augmented in atherosclerotic arteries (Table I).

As ergonovine and ergotamine contract vascular smooth muscle by an alpha adrenergic mechanism (20–23), we compared the effects of ergonovine with those of phenylephrine, an alpha agonist with selectivity for postjunctional receptors (24, 25). Fig. 2 shows that the concentration-response curves for phenylephrine in the two groups of arteries were similar. The threshold concentration was only slightly depressed in the atherosclerotic vessels, and the  $ED_{50}$  and maximum response were not different in the two groups (Table I). Thus, the supersensitivity of atherosclerotic arteries to ergonovine was not accompanied by a commensurate increase in the sensitivity to phenylephrine. This finding suggested that the observed ergonovine supersensitivity was not mediated predominantly by an alpha adrenergic mechanism.

In addition to stimulating alpha adrenergic receptors, ergot compounds may act on vascular serotonergic receptors (22, 23, 26). Accordingly, we have considered the possibility that the supersensitivity of atheroscler-

TABLE I  
Threshold Concentrations  $ED_{50}$  Values, and Maximal Responses in Aortic Strips  
from Normal and Cholesterol-fed Rabbits

	<i>n</i>	Threshold concentration	$ED_{50}$	A maximal response	B response to 34 mM KCl	A/B
			$\mu M$	$mg/mm^2$	$mg/mm^2$	
Ergonovine						
Control rabbits	9	$0.23 \pm 0.04 \mu M$	$2.0 \pm 0.4$	$255 \pm 38$	$186 \pm 36$	$1.37 \pm 0.11$
Cholesterol-fed rabbits	9	$0.51 \pm 0.18 pM$	$0.52 \pm 0.08$	$341 \pm 38$	$188 \pm 18$	$1.81 \pm 0.6$
<i>P</i> value		$<0.01$	$<0.01$	$<0.01$	NS	$<0.01$
Phenylephrine						
Control rabbits	9	$0.062 \pm 0.001 \mu M$	$0.44 \pm 0.03$	$347 \pm 43$	$186 \pm 36$	$1.86 \pm 0.12$
Cholesterol-fed rabbits	9	$0.01 \pm 0.002 \mu M$	$0.44 \pm 0.04$	$349 \pm 38$	$188 \pm 18$	$1.85 \pm 0.13$
<i>P</i> value		$<0.05$	NS	NS	NS	NS
Serotonin						
Control rabbits	7	$0.055 \pm 0.001 \mu M$	$0.25 \pm 0.02$	$184 \pm 23$	$186 \pm 24$	$0.99 \pm 0.05$
Cholesterol-fed rabbits	8	$810 \pm 60 pM$	$0.08 \pm 0.003$	$231 \pm 17$	$187 \pm 22$	$1.23 \pm 0.06$
<i>P</i> value		$<0.01$	$<0.05$	$<0.01$	NS	$<0.01$

Threshold concentration represents the minimal concentration of an agonist required to evoke a significant ( $P < 0.05$ ;  $t$  test for paired samples) increase in tone. A/B, value of the maximal response divided by the value of the response to KCl. *P* values were calculated on the basis of  $t$  tests for unpaired samples. Concentration-response relations for ergonovine and phenylephrine were studied in the same aortic strips as described in Methods. Experiments with serotonin were performed in additional groups of arteries.

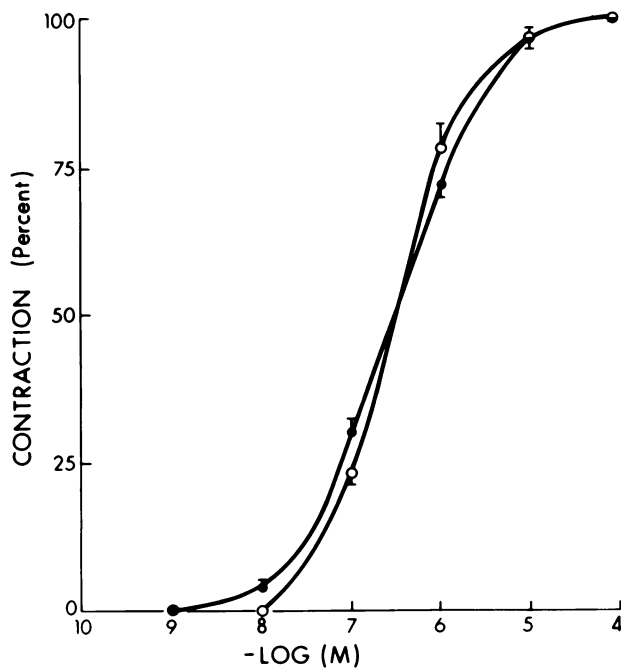


FIGURE 2 Concentration-response relations for phenylephrine in aortic strips from control (O) and cholesterol-fed (●) rabbits ( $n = 9$ ). Vertical bars represent 1 SE. Threshold concentrations,  $ED_{50}$ , and maximum responses derived from the same experiments are given in Table I.

otic aortae was mediated by a serotonergic mechanism. The concentration-response curves for serotonin in control and atherosclerotic arteries are shown in Fig. 3. The curve for atherosclerotic arteries was shifted to the left and exhibited significantly reduced values for the threshold concentration and the  $ED_{50}$  (Table I). In addition, the maximum response to serotonin was increased in the atherosclerotic group (Table I).

Increased responses of isolated arteries to an agonist may be related to structural vascular changes such as medial hyperplasia, or to a true supersensitivity of the smooth muscle cells, or to a combination of these factors (27). Pure supersensitivity produces a leftward shift of the agonist concentration-response curve but may not alter the maximum response. In the present experiments, the concentration-response relations for ergonovine and serotonin in atherosclerotic arteries exhibited depressed threshold concentrations and  $ED_{50}$  values, findings suggestive of supersensitivity. However, there was in addition an increase in the maximum responses, a result that may be attributed to structural changes. Structural alterations producing quantitative rather than qualitative changes in arterial contractility may be expected to exert similar effects on the maximum responses and  $ED_{50}$  values for different agonists. In this study, the ratio of the ergonovine threshold concentration to the phenylephrine thresh-

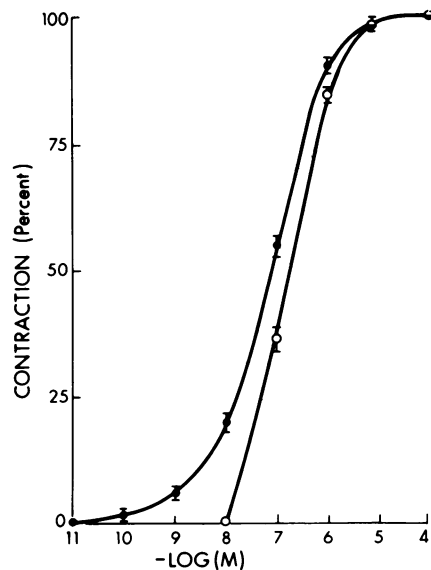


FIGURE 3 Concentration-response relations for serotonin in aortic strips from control (O,  $n = 7$ ) and cholesterol-fed (●,  $n = 8$ ) rabbits. Vertical bars indicate  $\pm$ SE. Threshold concentrations,  $ED_{50}$ , and maximum responses derived from the same experiments are shown in Table I.

old concentration was depressed in the atherosclerotic group ( $5.1 \cdot 10^{-13}/1.0 \cdot 10^{-8} = 5.1 \cdot 10^{-5}$ ) compared to the control group ( $2.3 \cdot 10^{-7}/6.2 \cdot 10^{-8} = 3.7$ ;  $P < 0.01$ ) (Table I). On the other hand, responses to KCl were virtually identical to the two groups of arteries (Table I). Maximum responses to ergonovine and serotonin in atherosclerotic arteries were significantly elevated when expressed with respect to the KCl responses (Table I). In contrast, maximum responses to phenylephrine related to the KCl responses were not significantly different in the two groups (Table I). The shifts in the threshold concentration, a parameter that should be insensitive to structural changes (27), and the significant changes in the response ratios in atherosclerotic arteries indicate that the altered responsiveness reflected in part true supersensitivity.

**Effects of antagonists.** To further delineate the altered responsiveness of atherosclerotic arteries to ergonovine and serotonin, experiments with antagonists were performed. To ascertain whether prazosin, phentolamine, and cyproheptadine affected resting arterial tone, the drugs were each tested in groups of four control and four atherosclerotic arteries after a standard equilibration period of 2 h. The antagonists in concentrations ranging between 0.01 and  $1 \mu\text{M}$  exerted no effect on resting tone, in agreement with previous reports (15, 28).

Fig. 4 depicts the effects of prazosin, an alpha adrenergic blocker with selectivity for postjunctional receptors (29), on arteries contracted with ergonovine. In control arteries,  $0.1 \mu\text{M}$  prazosin reduced arterial

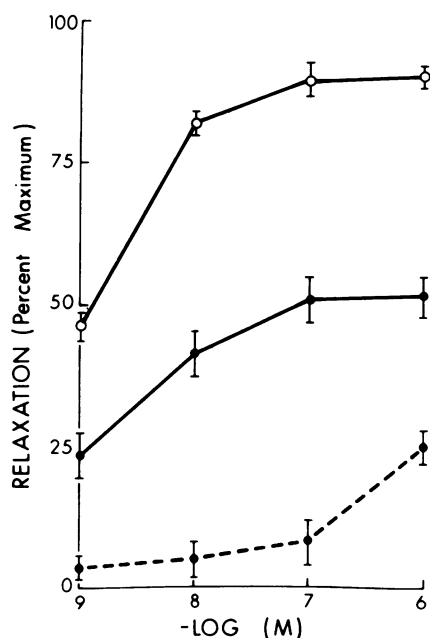


FIGURE 4 Relaxation of ergonovine-induced contractions by prazosin. The ordinate in this and in the subsequent figures indicates the percent reduction of the increase in tone produced by ergonovine (100% relaxation indicates a return to the tone before addition of ergonovine). Each arterial strip was first equilibrated with a selected ergonovine concentration. Subsequently, each strip was exposed to a single concentration of prazosin and allowed to reach a new equilibrium. Each data point represents the mean  $\pm$  SE of four to six experiments with four to six different strips. ○ — ○, control-1  $\mu$ M ergonovine; ● — ●, cholesterol-fed-1  $\mu$ M ergonovine; ● --- ●, cholesterol-fed-0.01  $\mu$ M ergonovine.

tone developed in response to 1  $\mu$ M ergonovine by 90%. In contrast, atherosclerotic arteries contracted by 1  $\mu$ M ergonovine relaxed only by 51% in response to 0.1  $\mu$ M prazosin, and no further relaxation was obtained with 1  $\mu$ M prazosin. In addition, contractions elicited by 0.01  $\mu$ M ergonovine were insensitive to the blocking effects of prazosin. Phentolamine, an alpha blocker that acts both on pre- and postjunctional alpha receptors (24, 29), was less potent than prazosin, but exerted qualitatively similar effects on arteries exposed to ergonovine (Fig. 5). With 1  $\mu$ M phentolamine, arterial contractions evoked by 1  $\mu$ M ergonovine were largely abolished in normal arteries, but reduced by only about 54% in atherosclerotic arteries. Furthermore, contractions induced in atherosclerotic vessels by a low concentration of ergonovine were only minimally blocked by phentolamine. Results with the two alpha adrenergic blockers indicate that contractions induced in atherosclerotic aortae by low concentrations of ergonovine were not mediated predominantly by an alpha adrenergic mechanism.

As ergot alkaloids may act on serotonergic receptors, we have examined the effects of the serotonergic

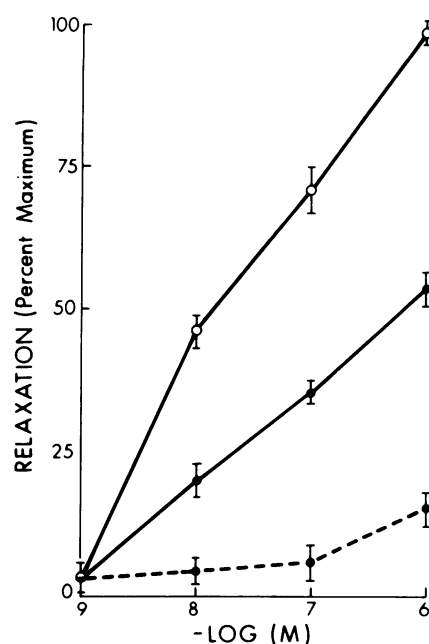


FIGURE 5 Relaxation of ergonovine-induced contractions by phentolamine. The experimental procedure was as described in the legend to Fig. 4. ○ — ○, control-1  $\mu$ M ergonovine; ● — ●, cholesterol-fed-1  $\mu$ M ergonovine; ● --- ●, cholesterol-fed-0.01  $\mu$ M ergonovine.

blocking agent cyproheptadine (22, 26) on ergonovine-induced contractions. In arteries stimulated with 1  $\mu$ M ergonovine, relaxations produced by 0.01 and 0.1  $\mu$ M cyproheptadine were significantly greater in atherosclerotic than in control arteries (Fig. 6). Moreover, contractions evoked in atherosclerotic arteries by 0.01  $\mu$ M ergonovine were largely inhibited by 0.1  $\mu$ M cyproheptadine (Fig. 6). This finding is in contrast to the modest relaxing effects of 0.1  $\mu$ M prazosin or 0.1  $\mu$ M phentolamine on these contractions (Figs. 4 and 5).

In additional experiments, concentration relations for serotonin with or without blocking agents were studied. After 2 h of incubation with phentolamine or prazosin (both  $n = 7$  and 0.1  $\mu$ M), contractions elicited in atherosclerotic arteries by up to 0.1  $\mu$ M serotonin were attenuated by less than 10% compared to contractions in atherosclerotic arteries ( $n = 7$ ) without alpha blockers. In contrast, with 0.1  $\mu$ M cyproheptadine as the inhibitor, contractions in atherosclerotic strips ( $n = 6$ ) in response to 0.1  $\mu$ M serotonin were completely abolished. On the other hand, 0.1  $\mu$ M cyproheptadine did not suppress the response of coronary arteries to 1  $\mu$ M phenylephrine, contractions with and without the blocking agent averaging  $246 \pm 30$  and  $258 \pm 34$  mg/mm<sup>2</sup> ( $n = 7$ ;  $P > 0.1$ ), respectively. With 1  $\mu$ M cyproheptadine, however, responses to 1  $\mu$ M phenylephrine were significantly inhibited

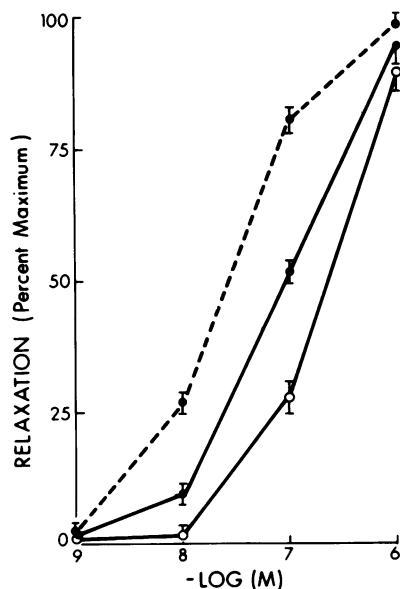


FIGURE 6 Relaxation of ergonovine-induced contractions by cyproheptadine. The experimental procedure was as described in the legend to Fig. 4. With  $10^{-8}$  M and  $10^{-7}$  M cyproheptadine relaxations of contractions induced by  $1 \mu\text{M}$  ergonovine were significantly different in control and atherosclerotic arteries ( $P < 0.05$ ;  $t$  test for unpaired samples).  $\circ$ — $\circ$ , control- $1 \mu\text{M}$  ergonovine;  $\bullet$ — $\bullet$ , cholesterol-fed- $1 \mu\text{M}$  ergonovine;  $\bullet$ --- $\bullet$ , cholesterol-fed- $0.01 \mu\text{M}$  ergonovine.

and averaged  $124 \pm 14 \text{ mg/mm}^2$  ( $n = 7$ ;  $P < 0.01$ ). Thus,  $0.1 \mu\text{M}$  cyproheptadine provided a selective serotonergic blockade, but higher concentrations of the drug had some alpha blocking activity.

In atherosclerotic arteries, alpha blockade with  $1 \mu\text{M}$  prazosin and serotonergic blockade with  $0.1 \mu\text{M}$  cyproheptadine each produced  $\sim 50\%$  inhibitions of the contractions induced by  $1 \mu\text{M}$  ergonovine (Figs. 4 and 6). To determine the effects of a blockade with the two drugs, atherosclerotic arteries stimulated with  $1 \mu\text{M}$  ergonovine were exposed simultaneously to  $0.1 \mu\text{M}$  prazosin and  $0.1 \mu\text{M}$  cyproheptadine. Combined blockade suppressed the contractions by  $91 \pm 4\%$  ( $n = 8$ ), suggesting that the alpha and serotonergic blocking effects of the drugs were additive.

As ergot alkaloids (ergotamine) may act on veins by stimulating prostaglandin synthesis (30) and as prostacyclin generation may be suppressed in aortae from cholesterol-fed rabbits (31, 32), we have considered the possibility that enhanced constrictor responses to ergonovine in atherosclerotic strips may reflect in part decreased prostacyclin-dependent vascular relaxation. After incubation with  $5.10 \mu\text{M}$  indomethacin, a cyclooxygenase inhibitor, threshold concentrations for ergonovine in normal ( $n = 5$ ) and atherosclerotic strips ( $n = 6$ ) averaged  $0.24 \pm 0.06 \mu\text{M}$  and  $0.59 \pm 0.15 \text{ pM}$ , respectively. In these same arteries,

$\text{ED}_{50}$  values in normal and diseased vessels were  $2.1 \pm 0.6$  and  $0.58 \pm 0.09 \mu\text{M}$ . These threshold concentrations and  $\text{ED}_{50}$  values did not differ significantly ( $P > 0.5$ ) from those obtained in the absence of indomethacin (Table I). Therefore, prostacyclin generation did not appear to play an important role in determining the supersensitivity to ergonovine.

## DISCUSSION

The present study indicates that aortae from rabbits fed a high-cholesterol diet are markedly supersensitive to the constrictor effects of ergonovine. In control aortae, contractions induced by ergonovine were effectively suppressed by alpha adrenergic blockade with prazosin or phentolamine. This finding confirms previous reports (20, 21) indicating that ergot alkaloids constrict blood vessels by stimulating alpha adrenergic receptors. In atherosclerotic arteries, on the other hand, contractions elicited by low concentrations of ergonovine ( $< 0.1 \mu\text{M}$ ) were not appreciably inhibited by alpha adrenergic blockers. However, cyproheptadine in a concentration that selectively antagonized serotonin largely suppressed these contractions. Thus, it appears that the supersensitivity of atherosclerotic arteries to ergonovine was mediated predominantly by a serotonergic mechanism. The fact that atherosclerotic arteries exhibited in addition a lowered threshold concentration and  $\text{ED}_{50}$  value for serotonin is consistent with an altered serotonergic responsiveness. Within the range of high ergonovine concentrations ( $> 0.1 \mu\text{M}$ ), contractions in atherosclerotic arteries were only partially blocked by cyproheptadine or alpha adrenergic blockade. However, in combination these agents largely suppressed the contractions, suggesting that within this range responses were mediated both by an alpha adrenergic and serotonergic mechanism.

Functional changes in atherosclerotic arteries may be attributed to alterations in vascular structure. However, nonspecific structural alterations such as smooth muscle hyperplasia or fibrosis should affect responses to all agonists to a similar extent (27, 33). In the present study, responses of atherosclerotic arteries to ergonovine were altered, whereas those to KCl or phenylephrine exhibited no or minor changes. In addition, the altered reactivity to ergonovine was associated with a marked suppression of the threshold concentration, a parameter that should be insensitive to structural changes (27). These findings strongly suggest that the altered responsiveness of atherosclerotic arteries did not result exclusively from nonspecific structural changes.

It has been demonstrated that ergotamine stimulates prostaglandin synthesis in canine saphenous veins (30), and that prostacyclin ( $\text{PGI}_2$ ) synthesis is suppressed

in aortae from cholesterol-fed rabbits (31). Therefore, we have considered the possibility that augmented contractions in response to ergonovine reflected in part a decreased prostacyclin-dependent relaxation of vascular smooth muscle. However, inhibition of prostaglandin synthesis with indomethacin had no apparent effect on the responses to ergonovine. Thus, it does not appear that the altered reactivity of atherosclerotic arteries to ergonovine was caused by a deficient synthesis of prostacyclin.

Accumulation of cholesterol in the membrane fraction of arteries constitutes one of the earliest demonstrable biochemical changes during atherogenesis (34). In recent studies (12, 13), we have shown that cholesterol in aqueous solution (1–100 pM) and human low density lipoprotein evoke dose-dependent augmentations in arterial tone. These findings suggest that acquisition of membrane cholesterol affects the contractile properties of arteries. It has been generally recognized that cholesterol is an important determinant of the structural and functional properties of biomembranes (35). Decreased membrane fluidity resulting from an acquisition of cholesterol may alter the distribution and function of membrane proteins (35). Thus, incorporation of cholesterol into membranes may alter the activity of membrane-bound enzymes (36, 37). Therefore, it is possible that altered responses to agonists observed in this study are related to a change in the lipid environment of the receptor molecules.

Recently, proliferation of arterial smooth muscle has been recognized to play a key role in the formation of atherosclerotic lesions. The present study provides for the first time evidence that smooth muscle in arteries undergoing atherosclerotic changes may in addition acquire an altered reactivity to specific vasoactive stimuli. The results support the hypothesis that altered vasomotion may contribute to arterial dysfunction in atherosclerosis.

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