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Effects of hypertension on hypercholesterolemia-induced changes in contraction of rabbit aorta and carotid artery

Robert S. Moreland [a,b,*], Alice H. Lichtenstein [a,1], Aram V. Chobanian [a]

[a] *Cardiovascular Institute, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118, USA*
[b] *Bockus Research Institute, Graduate Hospital, 415 South 19th Street, Philadelphia, PA 19146 USA*

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Abstract

Reactivity of aortic and carotid strips from control; hypertensive; hypercholesterolemic; and hypertensive/hypercholesterolemic rabbits was studied. Maximal stress was less in strips from hypertensive/hypercholesterolemic animals. Norepinephrine sensitivity was increased in the carotid artery from hypertensive/hypercholesterolemic animals (EC_{50}: 0.11 μM; 0.35 μM control). $CaCl_2$ sensitivity during norepinephrine-induced contractions was enhanced by hypertension and hypercholesterolemia (carotid EC_{50}: 0.10 mM; 0.38 mM control; aorta EC_{50}: 0.12 mM; 0.82 mM control). Similar results were obtained during membrane depolarization. 5-hydroxytryptamine sensitivity (EC_{50}: 0.15 μM carotid; 0.18 μM aorta) was decreased during hypertension (EC_{50}: 0.51 μM carotid; 1.13 μM aorta) and by hypercholesterolemia (EC_{50}: 1.76 μM carotid; 1.53 μM aorta). Our results support the hypothesis that hypertension and hypercholesterolemia increase vascular sensitivity by increasing Ca^{2+} permeability. Our results also suggest that hypertension and hypercholesterolemia selectively decrease 5-hydroxytryptamine-induced contractions.

Keywords: Aorta; Carotid artery; Norepinephrine; 5-HT (5-hydroxytryptamine, serotonin); Potassium chloride; Ca^{2+}; Cholesterol

1. Introduction

Hypertension is a well-defined risk factor for the development of atherosclerosis (for reviews, see Ross, 1986; Dzau, 1988; Chobanian, 1990). However, the mechanism(s) by which an increase in blood pressure results in an increase in the probability for the genesis of atherosclerosis is not well understood. As such, knowledge concerning how hypertension, hyperlipidemia, and most importantly the combination of the two pathophysiological states affect the normal physiological function of the vasculature is of interest.

Hypercholesterolemia is associated with significant changes in the sensitivity of the vasculature to contractile agents. Rabbits fed a high-cholesterol diet (Heric and Tackett, 1985; Maggi et al., 1985; Verbeuren et al., 1986; Hof and Hof, 1988; Hall et al., 1990; Du and Woodman, 1992; Merkel and Bilder, 1991; Fujiwara and Chiba, 1994) and rabbits with a genetic predisposition to atherosclerosis (Watanabe heritable hyperlipidemic rabbit; Kolodgie et al., 1990; Yokoyama et al., 1983; Wines et al., 1989) have an increased sensitivity to most vasoconstrictors. The changes in vascular function resulting from hypercholesterolemia are similar in direction to those shown to occur during hypertension that is increased sensitivity to contractile agents (Webb and Bohr, 1981). Whether hypertension and hypercholesterolemia produce these changes in vascular function by acting on the same pathway or by acting on different pathways that result in the same change is not known. Both hypertension and hypercholesterolemia of different etiologies increase membrane permeability (Holloway and Bohr, 1973; Jones and Hart, 1975; Menzoian et al., 1987; Strickberger et al., 1988; Bialecki and Tulenko, 1989; Tolins et al., 1992) resulting in increased Ca^{2+} influx, thereby augmenting vascular contractility.

The goal of this study was to determine whether hypertension augments the vascular alterations induced by hypercholesterolemia. Specifically, we were interested in determining whether the two pathophysiological states induced similar changes in vascular function or whether the

* Corresponding author. Tel.: (1) (215) 893-2378; fax: (1) (215) 893-7499.

[1] Present address: Lipid Metabolism Laboratory, USDA Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111, USA.

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two states affected different aspects of vascular regulation. Also of interest was whether the coexistence of the two states incrementally increased vascular response. In order to address these questions, we examined the vascular reactivity of aortic and carotid arterial strips from control; one-kidney, one clip renal hypertensive; cholesterol-fed hypercholesterolemic; and hypertensive/hypercholesterolemic rabbits.

2. Materials and methods

2.1. Animal model and characterization

Four groups (12 animals per group) of New Zealand white rabbits were used in this study: control; cholesterol-fed hypercholesterolemic; one-kidney, one clip renal hypertensive; and hypercholesterolemic/hypertensive rabbits. Control animals were maintained on standard lab chow and water ad libitum for 6 weeks. Cholesterol-fed animals were provided with a diet containing 1.5% cholesterol and 5.2% corn oil for 6 weeks. Hypertension was induced by removal of one kidney and placing a constrictive clamp on the renal artery of the remaining kidney; one-kidney, one clip renal hypertension has been previously described (Pott et al., 1986; Nickerson et al., 1992). To perform the surgical intervention, animals were given a sedative dose of xylazine (10 mg/kg i.m.) then anesthetized with ketamine HCl/acepromazine (35 mg/kg/0.75 mg/kg i.m.). The right kidney was exposed by a flank incision, the renal artery and vein tied, and the kidney removed. The left kidney was then exposed by a second flank excision and a non-occlusive sterling silver clip (0.8 mm inside diameter) was placed on the left renal artery. Following surgery the animals were either fed standard chow for 6 weeks (hypertensive) or after a recovery period of 3 days were fed a diet containing 1.5% cholesterol and 5.2% corn oil (hypercholesterolemic/hypertensive) for 6 weeks.

Systolic blood pressure of all animals was determined prior to and at weekly intervals during the 6-week time period. Systolic blood pressure was measured using a tail cuff plethysmograph as previously described by Kramsch et al. (1980) and Chobanian et al. (1989). Plasma cholesterol levels were determined prior to and at the end of the 6-week time period using an enzymatic technique (Sigma Diagnostics, St. Louis, MO, USA). Tissue cholesterol levels were measured using the method described by Rudel and Morris (1973). Carotid artery and aortic cholesterol levels were determined in the circumferential strips used for pharmacological study after the animals were killed.

2.2. Tissue preparation and isometric force recording

After 6 weeks of the appropriate intervention, the animals were killed by an injection of pentobarbital into the ear vein. The descending thoracic aorta, carotid arteries, and heart were rapidly removed, rinsed of blood in ice-cold physiological salt solution (PSS) and then placed in fresh ice-cold PSS. The aorta and carotid arteries were cleaned of fat and connective tissue, cut into circumferential strips approximately 2 mm in width and placed in a small PSS-containing petri dish. The circumferential strips were then chosen randomly, to avoid any bias that could be introduced by the presence or absence of an atherosclerotic lesion, and mounted on one end to a rigid clip attached to a micrometer for control of muscle length and on the other end to a Grass FT.03 force transducer connected to a Grass Model 7 polygraph. The mounted strips were placed in a PSS-filled water-jacketed muscle chamber maintained at 37°C, pH 7.4, and aerated with 100% O_2. The composition of the PSS was (in mM): 140 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.6 $CaCl_2$, 1.2 NaH_2PO_4, 2.0 3-[*N*-morpholino]-propanesulfonic acid (MOPS, pH 7.4), 5.0 D-glucose, and 0.02 Na_2EDTA.

The aortic strips were stretched to a pre-load of 1.0 g and the carotid arterial strips were stretched to a preload of 0.5 g. Preliminary studies demonstrated that this pre-load produced a tissue length that approximated the length for optimal active force development (L_o) in both tissues from all four animal groups. Both tissues were allowed to stress-relax and equilibrate for 60 min at which time they were contracted with 0.1 μM norepinephrine. Following complete relaxation from the norepinephrine stimulation, the strips were subjected to a series of stretch-stress relax cycles until a stable preload was maintained. The strips were then stimulated with 0.1 μM norepinephrine, relaxed, then restimulated to ensure stable force development of each vascular strip.

To ensure that all responses were the result of direct vascular smooth muscle cell stimulation and not modulated by simultaneous stimulation of endothelial cells, all strips were denuded of endothelium. This was performed by insertion of a wire rod into the lumen of the blood vessel prior to cutting of the circumferential strips. The adequacy of this technique was determined in all strips by verifying the complete abolition of acetylcholine-induced relaxation of a contraction in response to 0.1 μM norepinephrine.

2.3. Measurement of vascular reactivity

Two protocols were used to examine the reactivity of the vascular strips. The first protocol was to construct concentration-response curves by the cumulative addition of either norepinephrine alone, norepinephrine in the presence of 10 μM cocaine to block neuronal catecholamine uptake, or 5-hydroxytryptamine. The second protocol examined the Ca^{2+} sensitivity of contractions in response to either norepinephrine or KCl. The equilibrated strips were contracted by a maximal [norepinephrine] in the presence of Ca^{2+}-free PSS containing 0.2 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid (EG-

Table 1
Physiological characteristics of the four animal groups

Animal group	Initial body weight	Final body weight	Initial blood pressure	Final blood pressure	Heart weight/body weight
Control	3.29 ± 0.18	3.75 ± 0.15	109 ± 7.1	116 ± 6.5	1.74 ± 0.09
Cholesterol-fed	2.94 ± 0.06	3.42 ± 0.11	102 ± 2.3	104 ± 3.0	1.78 ± 0.06
Hypertensive	2.91 ± 0.07	3.29 ± 0.09	100 ± 5.2	151 ± 5.0 [a,c]	2.55 ± 0.12 [a,c]
Cholesterol-fed hypertensive	2.99 ± 0.05	3.53 ± 0.09	104 ± 5.4	153 ± 4.4 [a,c]	2.26 ± 0.11 [a,c]

Body weight in kg; blood pressure: systolic blood pressure in mm Hg; heart weight/body weight: ratio of left ventricular heart weight in g to body weight in kg. Values shown are means ± S.E. for 5–11 animals. [a] $P < 0.05$ as compared to control animals. [c] $P < 0.05$ as compared to cholesterol-fed animals.

TA) for 30 min to deplete intracellular Ca^{2+} stores. The strips were exhaustively rinsed with Ca^{2+}-free PSS then subjected to a 15-min exposure of a maximal [norepinephrine] in the presence of Ca^{2+}-free PSS containing 0.2 mM EGTA. The strips were rinsed 3 times for 5 min each in Ca^{2+}-free PSS. The strips were then stimulated with either 10 μM norepinephrine or 110 mM KCl-PSS (equimolar substitution for NaCl). $CaCl_2$ was added cumulatively to the tissue baths allowing steady-state force to develop between each addition.

2.4. Calculations and statistical analysis

Force values are expressed in units of stress (force normalized to cross-sectional area) using the following relationships: stress, in N/m^2 = (force, in g) × (9.807 × 10^{-3} N/g)/(cross-sectional area, in m^2) and cross-sectional area, in m^2 = (wet weight, in kg)/(length at L_0, in m) × (1,050 kg/m^3). The concentration of compound necessary to produce a half-maximal response (EC_{50}) was calculated using a Microsoft Excel macro as previously described (Fulginiti et al., 1993). Significant differences between means were determined using either the Student's *t*-test or single-factor analysis of variance. A value of $P < 0.05$ was taken as indicating significant differences.

2.5. Drugs

All vasoactive agents used in this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other compounds were of analytical grade or better.

3. Results

3.1. Animal characteristics

Basic physiological characteristics of the four groups of rabbits are presented in Table 1. All animals gained approximately the same percentage of initial body weight during the 6-week protocol; the increases ranged from 13–18%. Final systolic blood pressures of animals in the hypertensive and hypercholesterolemic/hypertensive groups were significantly elevated relative to blood pressures measured prior to surgery and to the two groups of normotensive animals. Rabbits from the hypertensive and hypercholesterolemic/hypertensive groups also had significantly elevated heart weight/body weight ratios as compared to the two normotensive groups. Cholesterol-feeding had no effect on systolic blood pressure or heart weight/body weight per se, or in combination with renal surgery.

Cholesterol-feeding in either the control or hypertensive rabbits significantly increased plasma, carotid artery, and aortic cholesterol levels (Table 2). Hypertension alone or in conjunction with cholesterol-feeding did not affect plasma cholesterol levels. Hypertension alone did not affect either carotid artery or aortic cholesterol levels (Table 2).

3.2. Vascular reactivity: norepinephrine

Figs. 1 and 2 show cumulative concentration response curves obtained by the addition of norepinephrine alone or

Table 2
Plasma and tissue cholesterol content in the four animal groups

Animal group	Plasma cholesterol	Carotid cholesterol	Aortic cholesterol
Control	47.3 ± 5.6	1.23 ± 0.27	1.35 ± 0.32
Cholesterol-fed	1900 ± 38.3 [a,b]	6.34 ± 4.9 [a,b]	9.55 ± 3.7 [a,b]
Hypertensive	36.4 ± 9.5	1.62 ± 0.21	1.65 ± 0.52
Cholesterol-fed hypertensive	1740 ± 40.4 [a,b]	6.58 ± 2.1 [a,b]	17.6 ± 6.1 [a,b]

Units of plasma cholesterol levels are mg/dl. Units of carotid and aortic cholesterol levels are mg/g wet weight tissue. Values shown are means ± S.E. for 5–9 determinations from different animals. [a] $P < 0.05$ as compared to control animals. [b] $P < 0.05$ as compared to hypertensive animals.

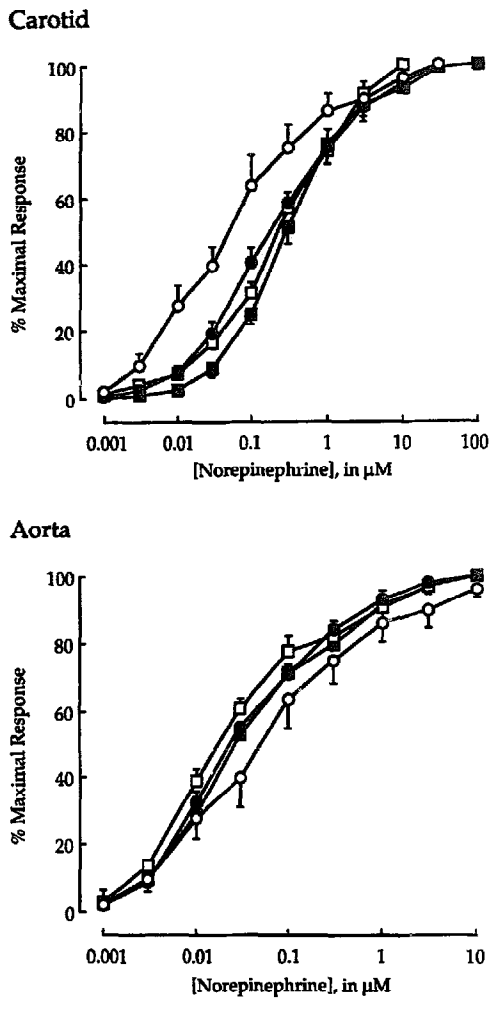

Fig. 1. Concentration-response relationship of carotid artery strips (top panel) and aortic strips (lower panel) from control (■), cholesterol-fed (●), hypertensive (□), and cholesterol-fed/hypertensive (○) rabbits to the cumulative addition of norepinephrine. All steady-state forces were normalized as a percentage of the maximal response of each individual vascular strip. Values shown are the means ± S.E. for at least 6 determinations.

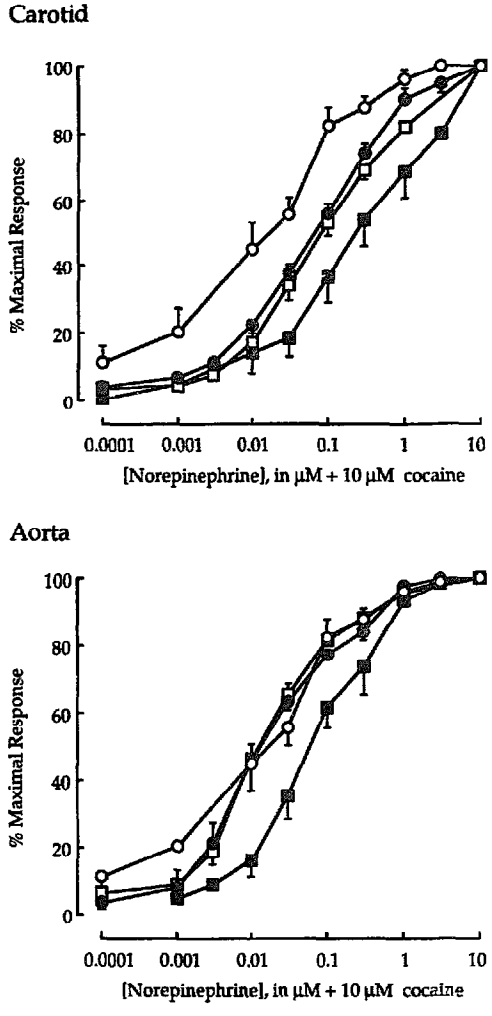

Fig. 2. Concentration-response relationship of carotid artery strips (top panel) and aortic strips (lower panel) from control (■), cholesterol-fed (●), hypertensive (□), and cholesterol-fed/hypertensive (●) rabbits to the cumulative addition of norepinephrine in the presence of 10 μM cocaine. All vascular strips were incubated in 10 μM cocaine for 15 min prior to the cumulative addition of norepinephrine. All steady-state forces were normalized as a percentage of the maximal response of each individual vascular strip. Values shown are the means ± S.E. for at least 5 determinations.

Table 3
Half-maximally effective concentrations (EC_{50}) of vasoactive compounds in carotid arterial strips in the four groups of animals

(A) Carotid	Control	Cholesterol-fed	Hypertensive	Cholesterol-fed hypertensive
Norepinephrine (μM)	0.35 ± 0.09	0.21 ± 0.04	0.23 ± 0.01	0.11 ± 0.03 [a-c]
Norepinephrine + cocaine (μM)	0.18 ± 0.09	0.08 ± 0.01	0.08 ± 0.02	0.02 ± 0.02 [a-c]
5-Hydroxytryptamine (μM)	0.15 ± 0.03	0.15 ± 0.02	0.51 ± 0.08 [a]	1.76 ± 0.76 [a-c]
$CaCl_2$ + norepinephrine (mM)	0.68 ± 0.07	0.22 ± 0.04 [a]	0.28 ± 0.03 [a]	0.10 ± 0.05 [a,b]
$CaCl_2$ + KCl (mM)	0.38 ± 0.03	0.35 ± 0.07	0.24 ± 0.03 [a]	0.11 ± 0.04 [a,c]
(B) Aorta	**Control**	**Cholesterol-fed**	**Hypertensive**	**Cholesterol-fed hypertensive**
Norepinephrine (nM)	30.9 ± 8.7	26.5 ± 4.2	19.3 ± 4.5	85.6 ± 11.1 [a-c]
Norepinephrine + cocaine (nM)	31.3 ± 1.7	13.6 ± 3.0 [a]	13.4 ± 3.4 [a]	17.1 ± 7.1 [a]
5-Hydroxytryptamine (μM)	0.18 ± 0.03	0.15 ± 0.03	1.13 ± 0.12 [a]	1.53 ± 0.44 [a,c]
$CaCl_2$ + norepinephrine (mM)	0.82 ± 0.04	0.29 ± 0.01 [a]	0.13 ± 0.04 [a]	0.12 ± 0.02 [a,c]
$CaCl_2$ + KCl (mM)	0.15 ± 0.02	0.18 ± 0.02	0.09 ± 0.02 [a]	0.08 ± 0.02 [a,c]

Dimension of EC_{50} values are as noted in parentheses. Values shown are means ± S.E. for 3–8 determinations. [a] $P < 0.05$ as compared to control animals. [b] $P < 0.05$ as compared to hypertensive animals. [c] $P < 0.05$ as compared to cholesterol-fed animals.

Table 4
Maximal stress development in aortic and carotid arterial strips in the four groups of animals

Carotid artery	Norepinephrine	Norepinephrine + cocaine	5-Hydroxytryptamine
Control	4.73 ± 0.47	5.08 ± 0.55	2.32 ± 0.09
Cholesterol-fed	5.12 ± 0.91	5.10 ± 0.82	2.81 ± 0.25
Hypertensive	5.59 ± 0.32	6.63 ± 0.61	2.49 ± 0.12
Cholesterol-fed hypertensive	2.83 ± 0.22 [a-c]	3.94 ± 0.48 [a-c]	1.21 ± 0.24 [a-c]
Aorta	**Norepinephrine**	**Norepinephrine + cocaine**	**5-Hydroxytryptamine**
Control	6.82 ± 0.56	7.15 ± 0.58	5.39 ± 0.42
Cholesterol-fed	7.90 ± 0.51	8.03 ± 0.21	4.41 ± 0.65
Hypertensive	5.49 ± 0.81	7.12 ± 0.52	4.33 ± 0.27
Cholesterol-fed hypertensive	3.85 ± 0.82 [a-c]	5.52 ± 0.91 [a,c]	2.13 ± 0.29 [a-c]

Units of stress are 10^4 N/m^2. Values shown are means ± S.E. for 3–8 determinations. [a] $P < 0.05$ as compared to control animals. [b] $P < 0.05$ as compared to hypertensive animals. [c] $P < 0.05$ as compared to cholesterol-fed animals.

in the presence of 10 μM cocaine to inhibit neuronal catecholamine uptake in carotid arterial and aortic strips from the four animal groups. The data shown in these figures were normalized as a percentage of the maximal response of each strip to norepinephrine in each of the four animal groups. The sensitivities of the strips to norepinephrine stimulation, defined as the concentration that elicits a half-maximal response (EC_{50}), are shown in Table 3 and the absolute levels of maximal stress developed in response to norepinephrine are shown in Table 4.

Carotid arterial sensitivity to norepinephrine was not affected by either hypercholesterolemia or hypertension alone but was increased by the combination of hypercholesterolemia and hypertension. In contrast, although aortic sensitivity to norepinephrine was also not affected by hypercholesterolemia or hypertension alone it was significantly reduced in hypercholesterolemic/hypertensive animals (Fig. 1, Table 3). The addition of cocaine increased norepinephrine sensitivity in all tissues except aorta from control animals (Fig. 2, Table 3). The addition of cocaine also resulted in a significant increase in norepinephrine sensitivity of aorta from hypercholesterolemic, hypertensive, and hypercholesterolemic/hypertensive animal as compared to control but had no effect on norepinephrine sensitivity of carotid arterial strips from the different animal groups.

Maximal levels of developed stress by carotid arterial or aortic strips in response to norepinephrine alone or in the presence of cocaine were not affected by either hypercholesterolemia or hypertension alone. The combination of hypercholesterolemia and hypertension significantly decreased the development of stress in both vascular preparations in response to norepinephrine in the presence and absence of cocaine (Table 4).

3.3. Vascular reactivity: 5-hydroxytryptamine

Fig. 3 shows cumulative concentration response curves obtained by the addition of 5-hydroxytryptamine. The data shown in these figures were normalized as a percentage of the maximal response of each strip to 5-hydroxytryptamine in each of the four animal groups. The sensitivities of the strips to 5-hydroxytryptamine are shown in Table 3 and the maximal levels of developed stress are shown in Table 4.

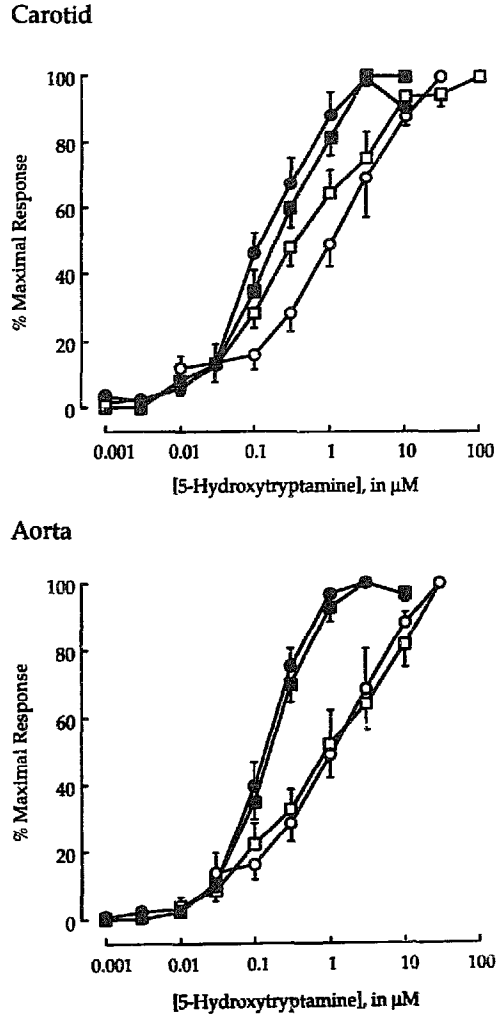

Fig. 3. Concentration-response relationship of carotid artery strips (top panel) and aortic strips (lower panel) from control (■), cholesterol-fed (●), hypertensive (□), and cholesterol-fed/hypertensive (●) rabbits to the cumulative addition of 5-hydroxytryptamine. All steady-state forces were normalized as a percentage of the maximal response of each individual vascular strip. Values shown are the means ± S.E for at least 7 determinations.

Cholesterol-feeding alone had no effect on carotid or aortic sensitivity to 5-hydroxytryptamine. Hypertension alone, however, significantly decreased 5-hydroxytryptamine sensitivity in both vascular preparations. The combination of the two pathophysiological states further decreased sensitivity to 5-hydroxytryptamine although the decrease was significant only in the carotid artery (Fig. 3, Table 3). Similar to the results obtained with norepinephrine, maximal stress development in response to 5-hydroxytryptamine was unchanged during hypercholesterolemia or hypertension but significantly decreased in both carotid and aortic strips from hypercholesterolemic/hypertensive animals (Table 4).

3.4. Vascular reactivity: $CaCl_2$

The sensitivity of the vascular strips from the four animal groups to $CaCl_2$ during norepinephrine-induced and KCl-induced contractions was determined and the results are shown in Figs. 4 and 5 and Table 3. Vascular sensitivity to $CaCl_2$ during both norepinephrine-induced and KCl-induced contractions was significantly increased by hypertension alone and the $CaCl_2$ sensitivity during norepinephrine-induced contractions by hypercholesterolemia alone in carotid arterial strips. $CaCl_2$ sensitivity was further increased in these tissues in response to either norepinephrine or KCl by a combination of the two interventions. Hypertension and hypercholesterolemia also increased the $CaCl_2$ sensitivity during norepinephrine-induced contractions of aortic strips. Aortic strips from hypertensive/hypercholesterolemic animals exhibited an increased $CaCl_2$ sensitivity during norepinephrine-induced contractions as compared to the sensitivity of aortic strips from animals with only hypercholesterolemia; the $CaCl_2$ sensitivity was not significantly different than that ob-

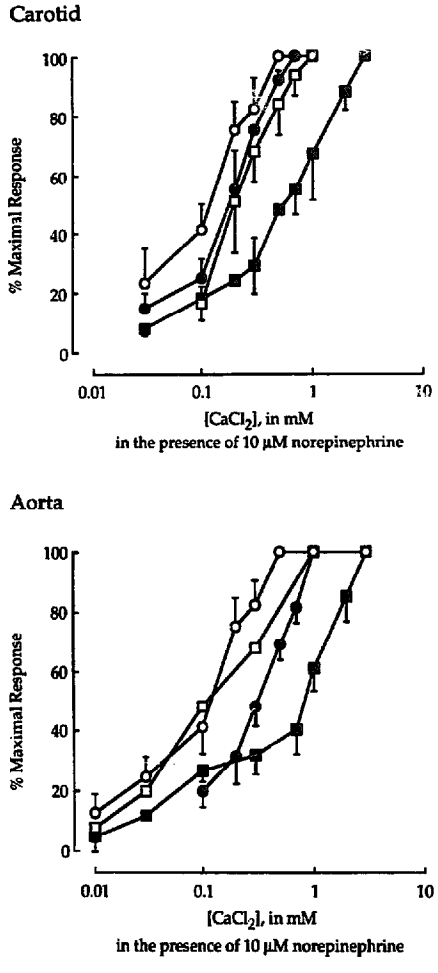

Fig. 4. Concentration-response relationship of carotid artery strips (top panel) and aorta strips (lower panel) from control (■), cholesterol-fed (●), hypertensive (□), and cholesterol-fed/hypertensive (○) rabbits to the cumulative addition of $CaCl_2$ during stimulation with 10 μM norepinephrine. All vascular tissues were depleted of cellular Ca^{2+} as described in Materials and methods then stimulated with norepinephrine. All steady-state forces were normalized as a percentage of the maximal response of each individual vascular strip. Values shown are the means ± S.E. for at least 3 determinations.

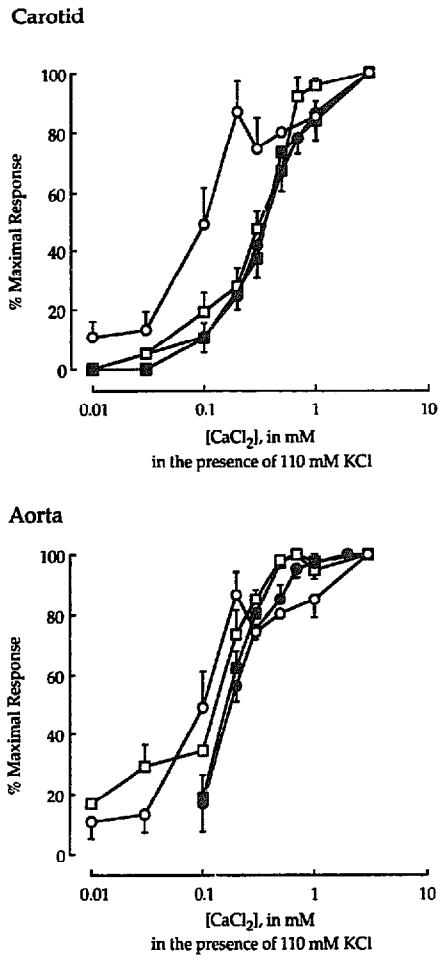

Fig. 5. Concentration-response relationship of carotid artery strips (top panel) and aortic strips (lower panel) from control (■), cholesterol-fed (●), hypertensive (□), and cholesterol-fed/hypertensive (○) rabbits to the cumulative addition of $CaCl_2$ during stimulation with 110 mM KCl. All vascular tissues were depleted of cellular Ca^{2+} as described in Materials and methods then stimulated with KCl. All steady-state forces were normalized as a percentage of the maximal response of each individual vascular strip. Values shown are the means ± S.E. for at least 4 determinations.

tained from animals with hypertension alone. Only hypertension increased the $CaCl_2$ sensitivity of aortic strips during membrane depolarization-induced contractions. $CaCl_2$ sensitivity during membrane depolarization in aortic strips from hypercholesterolemic was similar to that from control animals and the $CaCl_2$ sensitivity during membrane depolarization in aortic strips from hypercholesterolemic/hypertensive animals was similar to that from hypertensive only animals.

4. Discussion

The primary goal of this study was to determine if hypertension augments the hypercholesterolemia-induced changes in vascular reactivity; and if so do the two pathophysiological states affect similar components of vascular function. Alternatively, hypertension and hypercholesterolemia could alter vascular reactivity independently, suggesting that different components of vascular function are affected. The results of our experiments demonstrate that although hypertension and hypercholesterolemia affect some aspects of vascular function independently, they act synergistically to alter several fundamentally important parameters of vascular smooth muscle contraction.

The most consistent finding in this study was the significant depression of maximal stress development in vascular tissue from hypertensive/ hypercholesterolemic rabbits as compared to vascular strips from control, hypertensive, or hypercholesterolemic animals. Stress development was depressed in both carotid and aortic strips from the hypertensive/hypercholesterolemic rabbits in response to all agonists tested. Our results suggest that the reduction in the stress development observed is due to the combination of the two pathophysiological states. However, we can not rule out the possibility that the reduction may also be due simply to the way in which stress was calculated. Because of the method we used to calculate stress, which is force normalized to cross-sectional area of the tissue, it is significantly affected by the weight of the vascular strip. Cholesterol-fed rabbits develop atherosclerotic plaques in the vascular tissue as evidenced by increased cholesterol content and tissue weight. These factors alone would decrease the calculated value of stress. In spite of this, the maximal stresses developed in response to activation of vascular strips from hypercholesterolemic animals were not lower than that of vascular strips from control animals. It is important to note that the tissue cholesterol contents were determined in the same tissues that were used for the pharmacological studies and as such allow for a direct comparison. The cholesterol content of carotid arterial and aortic strips from hypertensive/ hypercholesterolemic rabbits were not significantly different than those from hypercholesterolemic rabbits although maximal stress development was depressed in strips from both vascular sources. Therefore, the presence of cholesterol alone or the effect of any additional tissue weight from atherosclerotic plaques on the calculation of stress cannot account for the depressed contractile ability of the vascular smooth muscle cells within the carotid artery or aorta from hypertensive/ hypercholesterolemic animals.

The maximal levels of stress developed by the carotid or aortic strips in response to agonist-stimulation were not affected by either hypertension or hypercholesterolemia alone. These results are consistent with previous reports demonstrating no change (Heric and Tackett, 1985; Verbeuren et al., 1986) or only a transient change (Du and Woodman, 1992) in maximal force generated by vascular tissues from cholesterol-fed male rabbits. In contrast, results of studies using aortic rings from Watanabe heritable hyperlipidemic rabbits have shown increases in maximal levels of force in response to histamine and 5-HT stimulation but decreases in adrenergic stimulation (Wines et al., 1989; Kolodgie et al., 1990). The augmented response to histamine and 5-HT (Kolodgie et al., 1990) and the decreased response to adrenergic stimulation (Du and Woodman, 1992) were independent of the endothelium and as such were apparently the result of a specific change in the vascular smooth muscle cell. Therefore, differences in results appear to be related to the precise location along the aorta from which the vascular rings were taken, the age of the animal, or a potential difference in hereditary versus experimental atherosclerosis and not due to the presence or absence of the endothelial cells. Hypertension alone has also been shown not to affect maximal force development of vascular strips to various agonists (Pott et al., 1986; Hall et al., 1990). These data and our results taken together suggest that hypertension and hypercholesterolemia act in a synergistic manner to depress contractility.

Precisely how these two pathophysiological states act to reduce contraction is not known. Both atherosclerotic lesions and hypertension induce medial thickening of blood vessels resulting in an increase in smooth muscle cellular mass and a narrowed lumen (Mulvany et al., 1978; Schwartz et al., 1990). Medial thickening induced by hypertension is usually characterized by an organized increase in smooth muscle cells oriented perpendicular to the long axis of the vessel (Nickerson et al., 1992). In contrast, medial thickening associated with atherosclerotic lesions is associated with an increase in smooth muscle cells that do not appear to have any apparent orientation and therefore would not contribute to force generated circumferentially in the vessel wall (Ross, 1986; Sreeharan et al., 1986). It is possible that all medial thickening in response to the combination of hypertension and hypercholesterolemia is characterized by smooth muscle cells not oriented circumferentially and therefore although the tissue is thicker and contains more smooth muscle cells, productive force generation is in fact less than that produced by a control blood vessel.

One potential mechanism by which maximal stress could be reduced in response to agonist activation of the

vascular strips is a decrease in receptor number as has been shown for glucocorticoid and endothelin receptors in response to atherosclerosis (Winkles et al., 1993; Sato et al., 1995). If this were the case, however, then the combination of hypertension and hypercholesterolemia must act synergistically to decrease receptor number since each state alone had no effect on maximal stress development. Another interesting possibility to account for the reduction in stress developed in vascular strips from the hypertensive/ hypercholesterolemic animals is an elevation in nitric oxide synthase content in the vascular smooth muscle cell. Verbeuren and his colleagues (1993) presented evidence to suggest that aortas of rabbits fed a high-cholesterol diet for 30 weeks contained a significantly elevated content of the inducible isoform of nitric oxide synthase. Therefore, for any given level of agonist activation, stress would potentially be less due to the presence of the potent relaxing compound, nitric oxide. Similar, however, to the potential role for a decrease in receptor number, since our tissues did not demonstrate reduced stress when obtained from hypercholesterolemic animals, the combination of hypertension and hypercholesterolemia must act synergistically to induce nitric oxide synthase. It is interesting to speculate that in our study in which the animals were fed a high-cholesterol diet for only 6 weeks as compared to 30 weeks in the study by Verbeuren et al. (1993), hypertension did in fact exacerbate the pathogenesis of the hypercholesterolemia and decrease the time required for induction of nitric oxide synthase.

The effect of hypertension, hypercholesterolemia, or the combination of both pathophysiological states on the sensitivity of carotid and aortic strips to vasoactive agents can be divided into two general effects, increased sensitivity to norepinephrine and decreased sensitivity to 5-HT. The carotid artery from hypercholesterolemic/hypertensive rabbits exhibited an increased sensitivity to norepinephrine both in the absence and presence of cocaine to block neuronal uptake of the catecholamine. Norepinephrine sensitivity in carotid strips from either hypercholesterolemic or hypertensive animals tended to be enhanced but the differences were not significant. The finding that the combination of the two pathophysiological states significantly enhanced norepinephrine sensitivity, whereas the single interventions did not, suggests that hypercholesterolemia and hypertension may act on similar pathways to alter norepinephrine sensitivity, at least in the rabbit carotid. This suggestion is further substantiated by the finding that either hypertension or hypercholesterolemia increased the sensitivity of the vessel to extracellular $CaCl_2$ during a norepinephrine contraction; the sensitivity was further increased by the combination of hypertension and hypercholesterolemia. norepinephrine contractions in the presence of cocaine and the addition of $CaCl_2$ during a norepinephrine contraction also exhibited an increased sensitivity in aorta from hypercholesterolemic/hypertensive animals. In agreement with previous publications (Holloway and Bohr, 1973; Bialecki and Tulenko, 1989; Hall et al., 1990; Bohr et al., 1991) we propose that hypertension alone and hypercholesterolemia alone increase Ca^{2+} influx in rabbit vascular tissue. In addition, we can extend this information by demonstrating that the influence of the two pathophysiological states is additive resulting in a significantly enhanced level of Ca^{2+} sensitivity relative to that measured in response to either disease state alone.

Whether the two pathophysiological states act on voltage-dependent Ca^{2+} channels or on receptor-operated Ca^{2+} channels is difficult to discern. Ca^{2+} sensitivity during a norepinephrine-induced contraction is increased in carotid strips from either hypertensive or hypercholesterolemic rabbits; Ca^{2+} sensitivity during a KCl-induced contraction is only enhanced in carotid strips from hypertensive rabbits. However, as stated above, Ca^{2+} sensitivity is further enhanced by the combination of the two states during either KCl- or norepinephrine-induced contractions. Qualitatively similar findings were seen with aortic strips. We have obtained preliminary evidence to suggest that norepinephrine-induced contraction of carotid and aortic strips from all four animals groups are sensitive to 100 nM nifedipine, suggesting that any change in Ca^{2+} permeability may involve L-type dihydropyridine-sensitive Ca^{2+} channels (unpublished observations). We interpret these data to suggest that there is a general increase in Ca^{2+} permeability induced by the combination of hypercholesterolemia and hypertension and specifically there may be an increase in Ca^{2+} influx through the dihydropyridine-sensitive Ca^{2+} channels.

Of potential importance was the finding that norepinephrine contractions in the absence but not in the presence of cocaine in aortic strips from hypercholesterolemic/hypertensive animals were less sensitive than aortic strips from control animals. Because aortas are in general sparsely innervated (Webb et al., 1981), cocaine would not be expected to have a large effect. However, if hypercholesterolemia plus hypertension increased either the level of innervation or catecholamine uptake activity, this could account for the differences in norepinephrine sensitivity. Our data do not address this possibility; we can only state that the combination of the two disease states significantly decreases norepinephrine sensitivity only in the absence of cocaine.

The combination of hypertension and hypercholesterolemia had significant effects on 5-HT receptors in both vessels. Hypertension significantly decreased vascular sensitivity to 5-HT in both tissues and this was further exacerbated by the addition of a high-cholesterol diet. Hypercholesterolemia alone had no effect on sensitivity to 5-HT. Several investigators have previously reported that vascular tissues from cholesterol-fed or Watanabe heritable hyperlipidemic rabbits exhibit increased sensitivity to 5-HT. Yokoyama and colleagues (1983) showed that aortic sensitivity to serotonin was significantly enhanced whereas carotid artery sensitivity was unchanged to 5-HT in Watan-

abe heritable hyperlipidemic rabbits. We do not know the reason for this difference but one potential explanation is the state of the endothelium in the respective vascular preparations. It has been proposed that enhanced sensitivity to 5-HT may be due to a decrease in release of endothelial dependent relaxing factor (Shimokawa and Vanhoutte, 1989). However, our vascular strips are devoid of the endothelium and although not explicitly discussed, we assume that the arterial strips used by Yokoyama et al. (1983) were also denuded. Our results are similar to those reported by Heric and Tackett (1985) who demonstrated that aortic strips, presumably endothelial-denuded, from rabbits fed a high-cholesterol diet for 6 weeks or longer exhibit no difference in sensitivity to 5-HT. It is interesting to note that Heric and Tackett (1985) presented evidence to suggest that sensitivity to 5-HT is enhanced in aortic strips from animals fed a high-cholesterol diet for only 4 weeks. These findings are consistent with work from Merkel et al. (1990) in which they demonstrated an enhanced sensitivity to 5-HT in aortic strips with an intact endothelial lining from rabbits fed a diet which produced only a modest increase in serum cholesterol as compared to the marked increases in our cholesterol-fed rabbits. The data suggest that the duration of cholesterol feeding and the final level of serum cholesterol may have a profound influence on the vascular sensitivity to serotonergic agonists.

In summary, we have shown that the combination of one-kidney, one-clip renal hypertension and cholesterol-fed hypercholesterolemia has significant effects on vascular reactivity in the rabbit. One of the primary alterations in vascular function that occurs in response to hypertension and hypercholesterolemia is a dramatic reduction in the maximal stress generated in response to all agonists tested. We demonstrated that both hypertension and hypercholesterolemia increase vascular sensitivity to norepinephrine and more importantly to $CaCl_2$ during either agonist or membrane depolarization-induced contractions. Therefore, our results are consistent with the hypothesis that hypertension and hypercholesterolemia increase membrane Ca^{2+} permeability resulting in an increased sensitivity to Ca^{2+} during contractile stimulation. The combination of the two pathophysiological states augments the increase in membrane permeability, suggesting that hypertension and hypercholesterolemia affect similar pathways in excitation-contraction coupling of vascular muscle.

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