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Changes in mechanical responses of vascular smooth muscles to acetylcholine, noradrenaline and high-potassium solution in hypercholesterolemic rabbits

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1 Mechanical responses produced by high potassium solution (high-[K]), noradrenaline (NA) and acetylcholine (ACh) were observed in the thoracic aorta and the main pulmonary artery isolated from control and hypercholesterolemic rabbits.

2 In the hypercholesterolemic rabbit, these tissues showed increased contractile sensitivity to high-[K], in comparison with those from the control rabbit. In tissues from the control rabbit, mechanical removal of the endothelium did not change the contraction to high-[K].

3 The amplitudes of the contractions produced by NA were decreased in tissues from the hypercholesterolemic rabbit, while they were enhanced after removal of the endothelium in tissues from the control rabbit.

4 The endothelium-dependent relaxant response to ACh observed in tissues from the control rabbit was diminished, abolished or changed to a contraction in tissues from the hypercholesterolemic rabbit.

5 It is concluded that in vascular tissues from the rabbit, an increase in contractile sensitivity to high-[K], a decrease in sensitivity to NA and dysfunction of the endothelium to ACh are induced by feeding a high-cholesterol diet and these changes in the vascular tissues may thus be involved in the hypertension observed during diet-induced atherosclerogenesis.

Introduction

During atherosclerogenesis, morphological, physiological or biochemical changes in blood vessels, such as increases in thickness and stiffness of vascular walls (Imai *et al.*, 1966; Parker & Odland, 1966; Cox & Detweiler, 1979), decreases in selective permeability of the endothelial layer to lipoproteins (Shimamoto, 1972) or increases in arterial permeability to albumin (Chobanian *et al.*, 1983) have been observed.

Pathological changes in the arterial wall, associated with atherosclerosis, are induced in laboratory animals fed a high-cholesterol diet (Roberts & Straus, 1965). In hypercholesterolemic rabbits, morphological or functional changes of vascular tissues have been noted (Imai *et al.*, 1966; Parker & Odland, 1966; Goode *et al.*, 1977; Henry & Yokoyama, 1980; Eldor *et al.*, 1982; Weigensberg *et al.*, 1982; Ingerman-Wojenski *et al.*, 1983). An increase in sensitivity of vascular smooth muscles to ergonovine (Henry & Yokoyama, 1980) or to 5-hydroxytryptamine (Yokoyama *et al.*, 1983) has also been observed.

We investigated the changes in the mechanical responses of arterial smooth muscles to some vasoactive agents in rabbits fed a high-cholesterol diet. The thoracic aorta and the main pulmonary artery were chosen, because the former is the most sensitive and the latter the most resistant elastic artery to a high-cholesterol diet (Constantinides, 1965).

Methods

Albino rabbits of either sex, aged 4–5 weeks (weighing between 300–500 g) were kept in individual cages and given sufficient water and food daily for a period of 10–12 weeks. One group of 40 rabbits was fed normal laboratory food (Oriental MF-4) containing 1% cholesterol (hypercholesterolemic rabbit), and a second group of 30 rabbits was maintained on normal laboratory food only (control). Body weights were measured twice a week. At the time of the experiments, mean body weights of the control and the hypercholesterolemic rabbits were 3.0 ± 0.3 kg ($n = 30$) and 3.5 ± 0.7 kg ($n = 40$), respectively and the serum cholesterol levels were 29–50 mg dl^{-1} and 980–1800 mg dl^{-1}, respectively. General pathological changes in vascular and non-vascular tissues of the

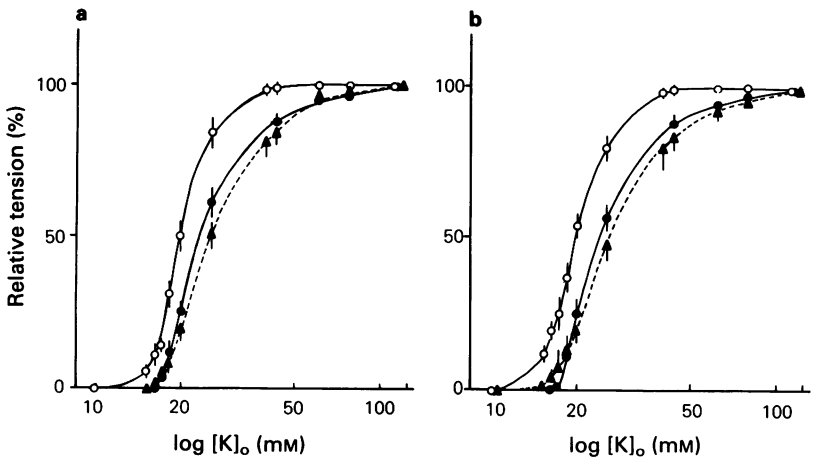

Figure 1 The dose-response relationship of the high-potassium induced contraction in aorta (a) and pulmonary artery (b) isolated from control (●, endothelium intact, ▲, endothelium removed) and hypercholesterolemic (○) rabbits. Amplitudes of contraction were expressed relative to those produced by 118 mM potassium solution in each experiment. Each point shows the mean ($n = 5-11$) and vertical lines indicate s.d.

hypercholesterolemic rabbit were as described by Constantinides (1965).

The descending thoracic aorta and the main pulmonary artery were taken from rabbits killed by bleeding from the femoral artery after being anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). The isolated tissues were cleaned of surrounding connective tissues, cut into rings (1.5–2.0 mm in width) with scissors and transverse strips prepared by cutting across the ring (1.2–1.5 cm in length). During preparation, great care was taken not to damage the endothelium or overstretch the tissue.

A pair of the strips was suspended parallel to each other in a 1 ml organ bath and isometric tension was recorded via a force transducer (Nihon Kohden, FD pick-up, TB-621T). The tissues were perfused with warmed (35.5°C) Krebs solution, at a flow rate of 3 ml min^{-1} using a perfusing pump (Tokyorikakikai, PO-1).

After incubation for 1 h without tension, a tension of 30–50 mg was applied to each tissue for at least 1 h before exposure to 118 mM potassium solution for 5 min every 30 min until the amplitude of the contraction reached a constant value. All these procedures usually required 3–4 h.

The endothelium was removed by gentle rubbing with a cotton ball moistened with Krebs solution, according to the method of Furchgott & Zawadzki (1980).

The aorta and pulmonary arteries from hypercholesterolemic rabbits were fixed in 10% formalin, embedded in paraffin and then stained by haematoxylin-eosin (Luna, 1968). Light microscopic examination confirmed the presence of the endothelium in the tissues before and after each experiment.

The ionic composition of the Krebs solution was as follows (mM); Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134, glucose 11.5. The solution was bubbled with 5% CO_2 in O_2, and the pH of the solution was maintained at 7.2–7.4. High-potassium solution was prepared by replacing NaCl with KCl.

Drugs used were acetylcholine chloride (Daiichi Pharm. Co.), noradrenaline hydrochloride (Sigma), atropine sulphate (Tanabe Pharm. Co.) and guanethidine sulphate (Tokyo Kasei Pharm. Co.). All drugs were dissolved in Krebs solution just before use.

Experimental values are expressed by mean value ± s.d. Statistical significances were tested by Student's t test, and probabilities of less than 5% ($P < 0.05$) were considered significant.

Results

High-[K]-induced contraction

Figure 1 shows the dose-response relationship of the effects of high-[K] on the aorta and main pulmonary artery from control and hypercholesterolemic rabbits. The perfusing solution contained 5 μM guanethidine, to eliminate possible involvement of the release of adrenoceptor agonists by high-[K]. Responses are expressed as a percentage of the amplitude of the maximum contraction produced by 118 mM [K].

In tissues isolated from the hypercholesterolemic rabbit, the dose-response relationship of the high-[K]-induced contractions shifted to the left of the control

Table 1 ED_{50} values for contraction produced by high-[K] (A) and noradrenaline (B) in the aorta and pulmonary artery from control and hypercholesterolemic rabbits

	Control	*Hypercholesterolemic*
A *High-[K]*		
Aorta	23.5 ± 1.9 mM (n = 5)	20.3 ± 1.9 mM* (n = 11)
Pulmonary artery	24.1 ± 2.7 mM (n = 5)	19.4 ± 1.6 mM* (n = 9)
B *Noradrenaline*		
Aorta	40 ± 2.1 nM (n = 9)	80 ± 3.1 nM* (n = 6)
Pulmonary artery	120 ± 0.8 nM (n = 9)	770 ± 5.0 nM* (n = 7)

The ED_{50} value was obtained from dose-response curves to either high-[K] or NA in each tissue. Data are mean ± s.d. (n = number of observations). * Significantly different from the control ($P < 0.05$).

curve with a significant lowering of the minimum concentration of [K] required to produce a contraction (13 mM cf. control, 17 mM), and also the concentration of [K] required to produce the maximum contraction (39.2 mM cf. control, 118 mM). Concentrations of K above 39.2 mM produced similar amplitudes of contractions both in the aorta and pulmonary artery.

The ED_{50} values for [K] were significantly decreased in both the aorta and pulmonary artery from the hypercholesterolemic rabbit compared with those from controls (Table 1).

The removal of the endothelium did not modify the contractile responses of the aorta and the pulmonary artery from control rabbits to high-[K] (Figure 1).

Noradrenaline-induced contractions

In tissues from both control and hypercholesterolemic rabbits, contractions were elicited by concentrations of NA above 10 nM, and the amplitude of contraction increased in a dose-dependent manner, to reach a maximum of about 120% (aorta) and 100% (pulmonary artery) of the 118 mM-[K]-induced contraction at 10 μM NA (Figure 2). The ED_{50} values were significantly lower in tissues from the control rabbit than in those from the hypercholesterolemic rabbit (Table 1).

After removal of endothelium in control tissues, the maximum contractile responses in both the aorta and pulmonary artery were significantly inreased (Figure 2).

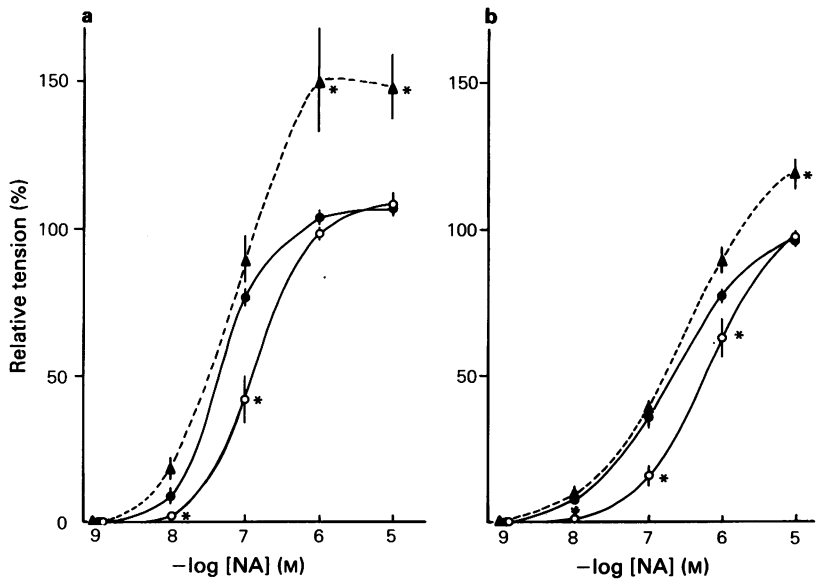

Figure 2 The dose-response relationship of the noradrenaline (NA)-induced contractions in aorta (a) and pulmonary artery (b) isolated from control (●, endothelium intact, ▲, endothelium removed) and hypercholesterolemic (○) rabbits. Amplitudes of contraction were expressed relative to those produced by 118 mM potassium solution in each experiment. Each point shows mean (n = 6–9) and vertical lines indicate s.d. * Significantly different from the control.

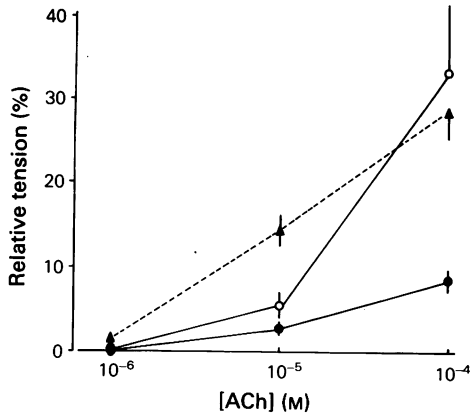

Figure 3 Dose-response relationship of the acetylcholine (ACh)-induced contractions in aorta isolated from the control (●, endothelium intact; ▲, endothelium removed) and hypercholesterolemic (○) rabbits. Amplitudes of contraction were expressed relative to those produced by 118 mM potassium solution. Each point shows mean (n = 5–8) and vertical lines indicate s.d.

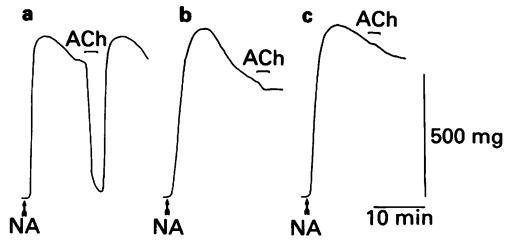

Figure 4 Typical recordings of the effects of acetylcholine (ACh; 100 nM) on noradrenaline (NA, 1 μM)-induced contractions in aorta from control (a) and hypercholesterolemic (b) rabbits. Response to ACh (100 nM) in tissue with damaged endothelium from the control rabbit is shown in (c). An arrow indicates where NA was applied.

Acetylcholine-induced contractions

ACh produced contractions in the aorta and the pulmonary artery in a dose-dependent manner. The threshold concentration of ACh was 10 μM in both tissues from control and hypercholesterolemic rabbits. The amplitudes of the ACh-induced contractions were larger in tissues from the hypercholesterolemic rabbits than in those from the controls when expressed as a percentage of the contraction produced by 118 mM [K] solution.

In the aorta isolated from control rabbits, removal of the endothelium enhanced the amplitude of the contractions induced by ACh (Figure 3). Application of 10 μM ACh produced larger contractions in tissues without endothelium than in tissues with intact endothelium or those from the hypercholesterolemic rabbit, and 100 μM ACh contracted the tissues to the same extent as that seen in tissues from the hypercholesterolemic rabbit.

Atropine 1 μM, blocked all these responses to ACh.

Acetylcholine-induced relaxation

ACh produces an endothelium-dependent relaxation in vascular smooth muscle preparations precontracted with high-[K], NA or other vasoactive agents (Furchgott, 1983). Experiments were carried out to observe the effects of ACh on contractions produced by NA in aorta isolated from rabbits fed either normal or high-cholesterol diets (Figure 4).

The ACh-induced relaxation observed in control tissues (Figure 4a) was diminished, or (in 3 out of 20 tissues) reversed to a contraction, in tissues from the hypercholesterolemic rabbit (Figure 4b). In control tissues, removal of endothelium resulted in a disappearance of the ACh-induced relaxation (Figure 4c).

In aorta from hypercholesterolemic rabbits, addition of 1 μM ACh during elevation of the tension by 1 μM NA produced neither relaxation nor contraction in 2 preparations, while it produced relaxation in a third preparation (the amplitude being about 5% of that of the contraction to 118 mM [K]). After removal of the endothelium, ACh (1 μM) enhanced the NA-induced contraction in all three tissues. The amplitude of the ACh-induced contraction was 9–15% of that of the 118 mM [K]-induced contraction in the first 2 preparations and was about 5% in the third. These results suggest that in tissues from the hypercholesterolemic rabbit, the endothelium was still functioning, although at a reduced level.

The dose-response relationship of the effects of ACh on NA (1 μM)-induced contractions showed that in aorta from the control rabbit, the concentration of ACh required to produce the maximum relaxation was 1 μM, and below or above this concentration of ACh the amplitude of the relaxation was smaller (Figure 5). In tissues from the hypercholesterolemic rabbit, ACh at concentrations below 10 μM relaxed the NA-induced contractions; however, the amplitude of the relaxations was much smaller than that seen in tissues from the control rabbit. Application of 100 μM ACh enhanced the NA-induced contraction. In the pulmonary artery, ACh produced mechanical responses similar to those seen in the case of the aorta.

In aorta from control and hypercholesterolemic rabbits, the relaxant response to ACh was compared after the muscle tension had been elevated to various levels by applying NA or high-[K]. The amplitudes of the muscle tension were expressed relative to that of the 118 mM [K]-induced contraction, while those of

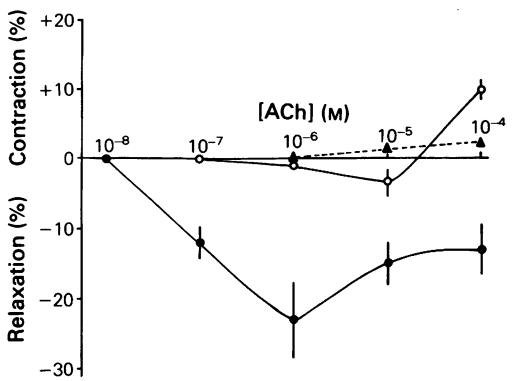

Figure 5 Dose-response relationship of the effects of acetylcholine (ACh) on noradrenaline (NA, 1 μM)-induced contraction in aorta isolated from control (●) and hypercholesterolemic (○) rabbits. (▲) Responses in tissues with damaged endothelium from the control rabbit. Amplitude of relaxation or contraction measured from the NA-induced tension was expressed relative to that produced by 118 mM potassium solution in each tissue. Each point shows the mean ($n = 10-15$) and vertical lines indicate s.d.

the ACh (100 nM)-induced relaxation or contraction were expressed relative to the tension at which ACh was applied. In the aorta from the control rabbit (Figure 6), amplitudes of the ACh-induced relaxant response were smaller when the tension was elevated by high-[K] than when it was elevated by NA. Nearly complete relaxation of the NA-induced contraction was elicited by 100 nM ACh, at any level of tension, except when the muscle was contracted to above 80% of that of the 118 mM [K]-induced contraction. In aorta from the hypercholesterolemic rabbit, the relaxant response to ACh was abolished during high-[K]-induced contraction, and was decreased in amplitude during NA-induced contraction, in comparison with that of the control.

In the pulmonary artery isolated from control rabbits (Figure 7), the ACh-induced relaxation was seen at any level of tension produced by either high-[K] or NA. However, the relaxant effects of ACh (100 nM) were weaker in the pulmonary artery than in the aorta (compare with Figure 6). The amplitude of the ACh-induced relaxation decreased gradually as the levels of tension by either high-[K] or NA were increased. In the pulmonary artery from the hypercholesterolemic rabbit, ACh did not modify the tension response to high-[K]. During NA-induced elevation of the tension, ACh produced both relaxation and contraction responses, but the relaxant response was smaller in comparison to those produced in control tissues.

The responses to ACh were reversible, and similar responses could be repeated several times, as long as the interval between the application of ACh exceeded 30 min. Addition of ACh at short intervals (usually below 20 min) decreased the subsequent response to ACh.

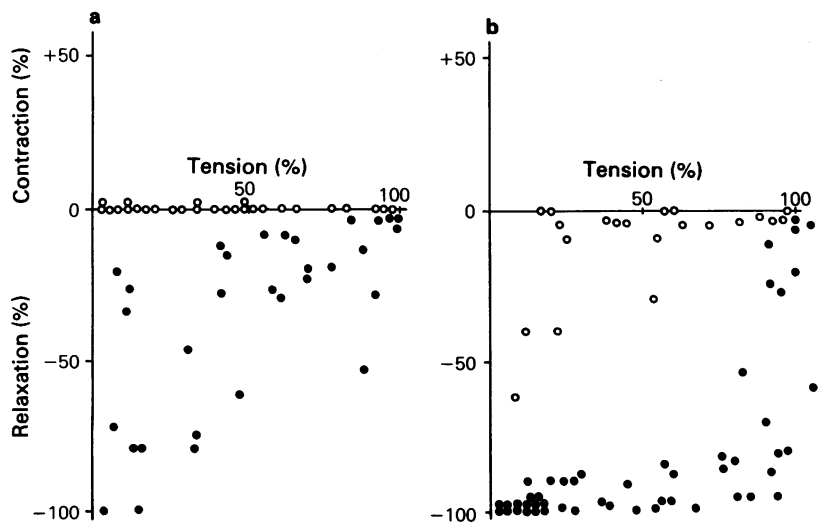

Figure 6 Effects of acetylcholine (ACh 100 nM) on contractions produced by high-[K] solution (a) and noradrenaline (NA) (b) in aorta from control (●) and hypercholesterolemic (○) rabbits. Amplitude of tension is expressed relative to contraction produced by 118 mM [K] solution, while amplitude of relaxation (negative value) or contraction (positive value) produced by ACh is plotted as a function of the tension (horizontal axis). Data from 12 tissues in the control rabbit and from 9 tissues in the hypercholesterolemic rabbit are summarized.

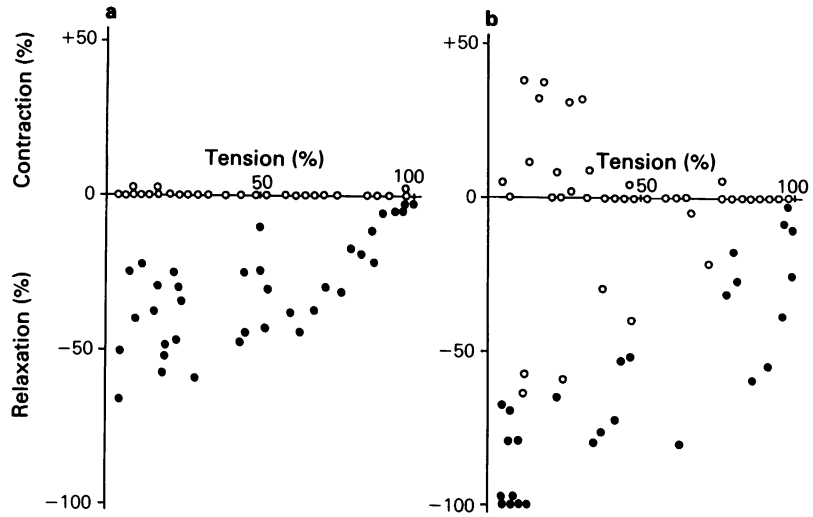

Figure 7 Effects of acetylcholine (ACh, 100 nM) on contractions produced by high [K] (a) and noradrenaline (NA) (b) in the pulmonary artery from control (●) and hypercholesterolemic (○) rabbits. Responses to ACh are plotted as described in legend of Figure 6.

Discussion

Endothelial cells play an important role in the relaxation of arterial smooth muscles produced by acetylcholine (ACh) or other vasoactive agents since these relaxant responses are absent in tissues denuded of endothelium (Furchgott & Zawadzki, 1980; Furchgott, 1983). We found that in tissues from the hypercholesterolemic rabbit, relaxation by ACh of high-[K]- or NA-induced contraction was decreased in amplitude, abolished completely or converted to a contraction, in comparison with those seen in the control rabbit. In hypercholesterolemic rabbits, morphological changes in the endothelial cell include disruption, swelling, vacuolization or irregularization (Parker & Odland, 1966; Goode *et al.*, 1977; Ingerman-Wojenski *et al.*, 1983). These lesions may constitute enough injury to diminish or terminate the synthesis of vascular relaxing substances in the cholesterol-induced atherosclerotic blood vessels. In vascular tissues from hypercholesterolemic animals, inhibition of prostaglandin synthesis, large increases in collagen synthesis or disturbances in enzyme production are observed (Haley *et al.*, 1977; Eldor *et al.*, 1982; Weigensberg *et al.*, 1982). These biochemical changes would reflect the general disturbances in metabolism occurring in vascular smooth muscle cells during atherosclerogenesis, and these disturbances may also affect synthesis of substances in the endothelial cells which induce vascular relaxation in response to ACh or other agents. The diminished or absence of a relaxant response to ACh in tissues from the hypercholesterolemic rabbit may therefore be attributed to a decrease in the function of the endothelial cells. Evidence that in tissues from the control rabbit, the ACh-induced relaxation was not seen after removal of the endothelium, supports this concept.

It is also possible that the endothelial cells are more fragile in tissues from the hypercholesterolemic rabbit than in those from the control. The endothelial cells can be removed easily by exposing the tissue to high [K] (40 mM) in the rabbit coronary artery (Griffith *et al.*, 1984) or by osmotic shock in the guinea-pig mesenteric artery (Bolton *et al.*, 1984). However, this was not the case in the aorta and the pulmonary artery from the hypercholesterolemic rabbit, because the presence of endothelial cells was histologically confirmed after the experiments. During cholesterol-induced atherosclerogenesis, the endothelial layer is separated from the medial layer due to an accumulation of cholesterol between these two layers (Constantinides, 1965). This may also contribute to a reduction of ACh-induced relaxation in tissues from the hypercholesterolemic rabbit.

In tissues from the control rabbit, the amplitude of the ACh-induced relaxation decreased when muscle tension had been elevated to above 70–90% of the maximum tension. This phenomenon was typically seen when muscle tension was elevated by high-[K]. The decrease in amplitude of the ACh-induced relaxation during a higher induced tension may by due to failure by endothelium-derived relaxing factor (EDRF) to decrease intracellular free Ca^{2+} concentrations which are elevated by high-[K] or by NA. In

vascular smooth muscles, intracellular free Ca^{2+} concentrations are below 10^{-7} M at rest and are above 10^{-5} M at maximum contraction produced by 118 mM-[K] (Itoh *et al.*, 1981). Therefore, intracellular Ca^{2+} concentrations up to 10^{-5} M may be reducible to below 10^{-7} M by EDRF. It is mainly extracellular Ca^{2+} that is involved in the production of the contraction to high [K] whereas with the NA-induced contraction both extra- and intracellular Ca^{2+} is used (Kuriyama, 1981). An increase in Ca^{2+} influx during high-[K] would counteract the EDRF-induced decrease in intracellular Ca^{2+} concentrations, and this would partly explain why the ACh-induced relaxation was smaller during elevation of tension by high-[K] than by NA.

In tissues from the hypercholesterolemic rabbit, sensitivity to high-[K] was increased in comparison with tissues from the control rabbit, i.e., there were decreases in the concentration of K required to produce the contraction and the ED_{50} value for the high-[K]-induced contraction. In tissues from the control rabbit, removal of the endothelium did not modify the sensitivity to high-[K]. Thus, these increases observed in tissues from the hypercholesterolemic rabbit may be due to changes in the properties of the smooth muscle itself, rather than as a result of injury to the endothelial cells. These alterations in the smooth muscle cells would be expected to occur when considering the morphological and biochemical changes observed in vascular tissues during cholesterol-induced atherogenesis, i.e., increase in foam cells (Haley *et al.*, 1977), decrease in cell proliferation (Weigensberg *et al.*, 1982) or decrease in $Na^+ K^+$-ATPase activity (Papahadjopoulos *et al.*, 1973). All these changes may modify the membrane properties of the smooth muscle cells so facilitating influx of Ca^{2+} ions into the cell (Yokoyama & Henry, 1979). There is evidence that in the main pulmonary artery of the pulmonary hypertensive rat, hypertrophy of the artery is accompanied by depolarization of the smooth muscle membrane (Suzuki & Twarog, 1982).

A constant release of EDRF has been suggested to occur in the rabbit aorta, from the evidence that hydroquinone, which blocks release of EDRF, increases tone under unstimulated conditions (Griffith *et al.*, 1984). This indicates that the NA-induced contractions occurring in isolated vascular tissues are underestimated due to the involvement of relaxation by EDRF. In the present experiment, the possible involvement of EDRF during the NA-induced contraction was also confirmed by removing the endothelium in the aorta and the pulmonary artery of the normal rabbit (Griffith *et al.*, 1984). However, in tissues from the hypercholesterolemic rabbit, responses to NA were decreased, in comparison with those from the control rabbit, although the amplitude of the maximum tension was not different in tissues from the two different types of rabbit. As sensitivity to high-[K] was increased by administration of a high-cholesterol diet, the reduction of the NA-induced contraction in tissues from the hypercholesterolemic rabbit may be due to a reduced sensitivity of α-adrenoceptors at the smooth muscle membrane.

In conclusion, vascular tissues of rabbit fed a high-cholesterol diet lose their dilating functions to ACh, presumably due to injury of the endothelium and also an increase in the sensitivity of the smooth muscle to depolarizing stimuli. In addition, the tension developed by NA was decreased by a high-cholesterol diet. Decrease in sensitivity to NA would result in unbalanced circulatory systems, due to disturbances adrenergic regulation of circulatory homeostasis. Thus, all these changes could help to explain the pathophysiology of vasospasm or hypertension in diet-induced atherosclerosis.

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References

BOLTON, T.B., LANG, R.J. & TAKEWAKI, T. (1984). Mechanisms of action of noradrenaline and carbachol on smooth muscle of guinea-pig anterior mesenteric artery. *J. Physiol.*, **351**, 549–572.

CHOBANIAN, A.V., MENZOIAN, J.O., SHIPMAN, J., HEATH, K. & HAUDENSCHILD, C.C. (1983). Effects of endothelial denudation and cholesterol feeding on *in vivo* transport of albumin, glucose, and water across rabbit carotid artery. *Circulation Res.*, **53**, 805–814.

CONSTANTINIDES, P. (1965). Experimental Atherosclerosis in the Rabbit. In *Comparative Atherosclerosis*, ed. Roberts, J.C. & Straus, R. pp. 276–290. New York: Harper and Row.

COX, R.H. & DETWEILER, D.K. (1979). Arterial wall properties and dietary atherosclerosis in the racing greyhound. *Am. J. Physiol.*, **236**(6), H790–H797.

ELDOR, A., FALCONE, D.J., HAJJAR, D.P., MINICK, C.R. & WEKSLER, B.B (1982). Diet-induced hypercholesterolemia inhibits the recovery of prostacyclin production by injured rabbit aorta. *Am. J. Pathol.*, **107**, 186–190.

FURCHGOTT, R.F. (1983). Role of endothelium in responses of vascular smooth muscle. *Circulation Res.*, **53**, 557–573.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* **288**, 373–376.

GOODE, T.B., DAVIES, P.F., REIDY, M.A. & BOWYER, D.E. (1977). Aortic endothelial cell morphology observed *in situ* by scanning electron microscopy during atherogenesis in the rabbit. *Atherosclerosis*, **27**, 235–251.

GRIFFITH, T.M., HENDERSON, A.H., EDWARDS, D.H. & LEWIS, N.J. (1984). Isolated perfused rabbit coronary artery and aortic strip preparations: the role of endothelium-derived relaxant factor. *J. Physiol.*, **351**, 13–24.

HALEY, N.J., SHIO, H. & FOWLER, S. (1977). Characterization of lipidladen aortic cells from cholesterol-fed rabbits. I. Resolution of aortic cell populations by metrizamide density gradient centrifugation. *Lab. Invest.*, **37**, 287–296.

HENRY, P.D. & YOKOYAMA, M. (1980). Supersensitivity of atherosclerotic rabbit aorta to ergonovine. *J. clin. Invest.*, **66**, 306–313.

IMAI, H., LEE, K.T., PASTORI, S., PANLILIO, E., FLORENTIN, R. & THOMAS, W.A. (1966). Atherosclerosis in rabbits. Architectural and subcellular alterations of smooth muscle cells of aortas in response to hyperlipemia. *Exp. mol. Pathol.*, **5**, 273–310.

INGERMAN-WOJENSKI, C.M., SEDAR, A.W., NISSENBAUM, M., SILVER, M.J., KLURFIELD, D.M. & KRITCHEVSKY, D. (1983). Early morphological changes in the endothelium of a peripheral artery of rabbits fed an atherogenic diet. *Exp. mol. Pathol.*, **38**, 48–60.

ITOH, T., KURIYAMA, H. & SUZUKI, H. (1981). Excitation-contraction coupling in smooth muscle cells of the guinea-pig mesenteric artery. *J. Physiol.*, **321**, 513–535.

KURIYAMA, H. (1981). Excitation-contraction coupling in various visceral smooth muscles. In *Smooth Muscle – An Assessment of Current Knowledge* ed. Bulbring, E., Brading, A.F., Jones, A.W. & Tomita, T. pp. 171–197. London: Edward Arnold.

LUNA, L.G. (1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd edition. New York: McGraw-Hill Book Company.

PAPAHADJOPOULOS, D., COWDEN, M. & KIMELBERG, H. (1973). Role of cholesterol in membranes effects on phospholipid-protein interactions, membrane permeability and enzymatic activity. *Biochim. biophys. Acta*, **330**, 8–26.

PARKER, F. & ODLAND, G.F. (1966). A correlative histochemical, biochemical and electron microscopic study of experimental atherosclerosos in the rabbit aorta with special reference to the myointimal cell. *Am. J. Pathol.*, **48**, 197–212.

ROBERTS, J.C. & STRAUS, R. (1965). *Comparative Atherosclerosis*. New York: Harper and Row.

SHIMAMOTO, T. (1972). New concept on atherogenesis and treatment of atherosclerotic diseases. *Jap. Heart J.*, **13**, 537–562.

SUZUKI, H. & TWAROG, B. (1982). Membrane properties of smooth muscle cells in pulmonary hypertensive rats. *Am. J. Physiol.*, **242**, H907–H915.

WEIGENSBERG, B.I., LOUGH, J. & MORE, R.H. (1982). Biochemistry of atherosclerosis produced by cholesterol feeding, thrombosis and injury. *Exp. mol. Pathol.*, **37**, 175–192.

YOKOYAMA, M. & HENRY, P.D. (1979). Sensitization of isolated canine coronary arteries to calcium ions after exposure to cholesterol. *Circulation Res.*, **45**, 479–486.

YOKOYAMA, M., AKITA, H., MIZUTANI, T., FUKUZAKI, H. & WATANABE, Y. (1983). Hyperreactivity of coronary arterial smooth muscles in response to ergonovine from rabbits with hereditary hyperlipidemia. *Circulation Res.*, **53**, 61–73.

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