Impairment of endothelium-dependent relaxation of aortas and pulmonary arteries from spontaneously hyperlipidemic mice (*Apodemus sylvaticus*)

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Abstract

We evaluated endothelial function of thoracic aortas and pulmonary arteries in a population of European wood mice (*Apodemus sylvaticus*), which exhibits hypercholesteromia. According to the plasma cholesterol level, mice were divided into two groups: hypercholesterolemic (AHL, total plasma cholesterol 200-300 mg/dl) and normocholesterolemic (ANL, total plasma cholesterol <200 mg/dl). Acetylcholine (ACh) caused endothelium-dependent relaxation of precontracted aortas and pulmonary arteries. Relaxation of the pulmonary artery is completely dependent on nitric oxide. This relaxation was inhibited in AHL pulmonary arteries. On the other hand, part of the ACh-induced relaxation of the thoracic aorta was resistant to N^{ω}-nitro-L-arginine (L-NNA). L-NNA-sensitive and -resistant relaxation to ACh were also inhibited in AHL aortas. Inhibition of endothelium-dependent relaxation of the aortas was correlated with total plasma cholesterol level. Endothelium-independent relaxation to sodium nitroprusside (SNP) was similar in AHL and ANL pulmonary arteries, but in the thoracic aorta of AHL mice, the sensitivity to SNP was slightly decreased, without a change in maximal response to SNP. No morphological change was observed in the aortas and the pulmonary arteries from AHL and ANL mice. Thus, AHL mice are valuable as a new experimental model to study the relation of hyperlipidemia to vascular disease since the endothelial function is impaired in these mild hyperlipidemic animals.

Key words: Endothelium; Hypercholesterolemia; Mice (*Apodemus sylvaticus*); Nitric oxide; Vascular reactivity

1. Introduction

Hypercholesterolemia is a major risk factor for development of atherosclerosis, which subsequently causes many cardiovascular diseases. Vascular endothelium-derived nitric oxide (NO) is thought to play a protective role against initiation and development of atherosclerosis. NO synthesized by endothelial nitric oxide synthase (eNOS) activates guanylate cyclase producing cyclic GMP, which inhibits vascular smooth muscle contraction, platelet activation and monocyte infiltration through activation of cyclic GMP-dependent protein kinase (Qian *et al*., 1999; Shimokawa, 1999; Hanafy *et al*., 2001; Shaul, 2003). Impairment of vascular endothelial functions, especially eNOS function, could be a causal factor for cardiovascular diseases such as hypertension, stroke and ischemic heart disease. It is believed that there is a relationship between hypercholesterolemia and endothelial dysfunction, as clinical (Bossaller *et al*., 1987; Creager *et al*., 1990; Zeiher *et al*., 1991) or experimental studies (Ibengwe and Suzuki, 1986; Jayakody *et al*., 1987; Osborne *et al*., 1989; Sellke *et al*., 1990; Verbeuren *et al*., 1990) have demonstrated that hypercholesterolemia impairs production and/or biological activity of NO, increasing vascular resistance in the early stage of atherosclerosis (Zeiher *et al*., 1991; Reddy *et al*., 1994). Moreover, an injury to the endothelium brings about migration of monocytes and accumulation of cholesterol in the subendothelial layer, leading to atherogenesis (Stocker and Keaney, 2004). Impairment of eNOS activity also increases counter-balanced products, reactive oxygen species (ROS), which contribute to development of atherosclerosis (Harrison, 1997; Yaghoubi *et al*., 2000). However, it is not fully understood whether chronic hyperlipidemia induces damage to endothelial cells, and if so, how it causes such impairment.

To clarify the relationship between hyperlipidemia and endothelial dysfunction, it is useful to study hyperlipidemic animals. For this purpose,

gene-manipulated hyperlipidemic animals have been generated, such as apolipoprotein E (apoE)-deficient mice (Plump *et al*., 1992; Zhang *et al*., 1992) or LDL receptor-deficient mice (Ishibashi *et al*., 1993). Besides, spontaneously hyperlipidemic (SHL) mice (Matsushima *et al*., 1999) or Watanabe heritable hyperlipidemic rabbits (Watanabe 1980) are also used as spontaneous animal models of hyperlipidemia. Here, we introduce a new hyperlipidemic model, the European wood mouse (*Apodemus sylvaticus*), which lives widely in the forests of southwestern Asia/Eurasia. Tsuchiya *et al*. (1992) have found that a population of these mice is hyperlipidemic when fed with common food. Administration of pravastatin, an HMG CoA reductase inhibitor (statin), to the hyperlipidemic (*Apodemus* hyperlipidemic, AHL) mice reduces plasma total cholesterol level (Tsuchiya *et al*., 1992). This contrasts with the usually employed experimental rats (*Rattus norvegicus*), in which statins cannot reduce plasma cholesterol (Tsujita *et al*., 1986; Yoshino *et al*., 1988). For this reason, it is expected that AHL mice are a good experimental model for studying the relationship between hyperlipidemia and atherosclerosis. To evaluate the suitability of *Apodemus sylvaticus* as a hyperlipidemic model, we examined the function of vascular endothelium to cause relaxation of the thoracic aortas and pulmonary arteries from mice exhibiting normolipidemia or hyperlipidemia.

2. **Materials and methods**

Experiments were conducted according to the Guide for the Care and Use of Laboratory Animals, Minami Kyushu University, Japan.

2.1. Animals and in vivo experiments

European wood mice of either sex (23–120 weeks old), which were

maintained as a closed colony at the Department of Bio-resources, Frontier Science Research Center, University of Miyazaki, Japan, under a controlled photoperiod (light, 8:00–20:00 h) and conditioned air supply (temperature 23.2°C; humidity 50.5%) were used. We defined young animals as 23–45 weeks old, and aged animals 50–120 weeks old. Separation of hypercholesterolemic mice was not successful because mating of male and female mice, both of which were hypercholesterolemic, did not necessarily give birth to hypercholesterolemic offspring. Therefore, we divided mice into two groups according to the plasma total cholesterol level. Animals with total cholesterol >200 mg/dl were grouped as AHL mice, and those with <200 mg/dl were grouped as *Apodemus* normolipidemic (ANL) mice. Mean body weight was 25.7±1.0 g (n=20) and 28.4±1.6 g (n=15) for ANL mice and AHL mice, respectively (Table 1).

Systolic and diastolic blood pressure and heart rate were measured in conscious animals by a noninvasive tail-cuff method (Softron BP-98A, Tokyo, Japan). Since European wood mice are very active, the animals were tamed by physical contact for several days before the measurement. Blood pressure and heart rate were measured several times in a rested condition and averaged. Plasma total cholesterol was measured using Dri-Chem 3500 (Fuji Film, Tokyo, Japan).

2.2. Tissue preparations

Mice were anesthetized with diethyl ether and killed by exsanguination. The main pulmonary artery and the thoracic aorta were dissected, and connective tissues were cleaned off in Krebs solution. The artery segment was cut into a ring 1.5–2 mm wide for tension measurement. When needed, the endothelium was removed by gently rubbing the intimal surface with a stainless steel wire.

2.3. Tension measurement

A tissue preparation was suspended in an organ bath containing 5 ml Krebs solution with the following composition (mM): 136.8 NaCl, 5.4 KCl, 2.5 $CaCl_2$, 1.0 $MgCl_2$, 11.9 $NaHCO_3$, 1.2 NaH_2PO_4 and 5.5 glucose (pH 7.4 when gassed with 95% O_2 and 5% CO_2) at 37°C. A change in tension was recorded isometrically under constant resting tension (0.3 g for aorta and 0.05 g for pulmonary artery) with a force–displacement transducer (Nihon-Kohden, Tokyo, Japan). After a 90 min equilibration period, the preparations were repeatedly contracted with hypertonic 60 mM KCl until the response became reproducible. Then, response to 5-hydroxytryptamine (5-HT) was observed to determine the concentration to induce 50-60 % of the maximum contraction. When a relaxation response to acetylcholine (ACh) or sodium nitroprusside (SNP) was measured, the preparation was precontracted with 5-HT at a concentration sufficient to induce 50–60% of the maximum contraction, and either drug was then cumulatively added. At the end of the experiment, 100 μM papaverine was added to obtain maximum relaxation in each preparation. Relaxation induced by papaverine was rated as 100%. The negative logarithm of concentration to cause 50 % inhibition (pD_2) was calculated from the individual dose-response relationship.

2.4. Histology

Thoracic aorta and pulmonary artery were fixed in buffered formalin (4%) and kept for 24 h at room temperature. Tissues were embedded in paraffin and processed for light microscopy using standard hematoxylin and eosin (HE) staining.

2.5. Statistics

Values are expressed as means±SEM. Student's *t* test was

performed for comparison of two values. ANOVA was performed for multiple comparisons followed by a post hoc test (Bonferroni/Dunn method). $P<0.05$ was considered to be statistically significant.

2.6. Drugs

ACh, N^{ω}-nitro-L-arginine (L-NNA), indomethacin, papaverine hydrochloride, SNP, forskolin and 5-HT were purchased from Sigma (St. Louis, MO, USA).

3. Results

3.1. Plasma total cholesterol level

We measured total plasma cholesterol in more than two hundred *A. sylvaticus* mice. About fifty mice showed cholesterol >200 mg/dl and 150 mice <200 mg/dl. Plasma total cholesterol in ANL and AHL mice was 124.8±5.5 mg/dl (n=20) and 219.5±4.8 mg/dl (n=15, $P<0.05$), respectively. There was no statistical difference in the plasma total cholesterol between young and aged mice (Table 1).

3.2. Blood pressure and heart rate

Blood pressure and heart rate measured in conscious condition is shown in Table 2. Systolic, mean or diastolic blood pressure was not different between ANL and AHL groups. Likewise, heart rate was also not different between two groups.

3.3. Vascular response in ANL and AHL mice

3.3.1. Thoracic aorta

The concentration of 5-HT needed to induce a contraction of ~60 % of the maximum response, which was obtained with 10 μM 5-HT, in the

thoracic aorta with intact endothelium from ANL and AHL mice was 0.29±0.08 μM (*n*=25) and 0.14±0.02 μM (*n*=23), respectively. These concentrations of 5-HT were not statistically different. Contraction induced by these concentrations were 96.1±8.5 mg (n=24) or 118.7±9.1 mg (n=25) for aortas from ANL or AHL mice, respectively, and there was no significant difference in the level of pre-contraction between both groups. ACh induced relaxation of aortas precontracted with 5-HT in a dose-dependent manner. ACh-induced relaxation of AHL aortas was significantly decreased compared with that of ANL aortas (Fig. 1). The pD_2 value of ACh in AHL aortas (7.20±0.12, *n*=25) was significantly smaller than that in ANL aortas (7.74±0.06, *n*=24, $P<0.05$). The maximum relaxation induced by ACh was also decreased in AHL aortas (74.7±4.6%, *n*=25) compared with that in ANL aortas (95.0±0.8%, *n*=24, $P<0.05$, Fig. 1 and Table 3). Bringing together the data from the two groups, the plasma total cholesterol was inversely correlated with ACh-induced relaxation of thoracic aorta (correlation coefficient *r*=0.73, Fig. 2).

Regarding the influence of aging, the maximum relaxation in response to ACh was inhibited in aortas from aged compared with young ANL mice (Fig. 3). On the other hand, there was no statistical difference in the ACh-induced maximum response between aged and young AHL mice (Fig. 3).

ACh-induced relaxation was abolished by endothelial denudation (data not shown). Pretreatment with the NO synthase inhibitor L-NNA (200 μM) for 15 min, which did not affect the 5-HT-induced contraction, completely abolished the relaxation to ACh of the thoracic aortas from AHL mice, whereas 23.9±3.6 % (*n*=24) of relaxation was resistant to L-NNA in ANL aortas (Table 3, Fig. 4). Even when 1 mM L-NNA was used to inhibit eNOS, we observed a similar L-NNA-resistant relaxation in

ANL aortas (28.9±7.7% of relaxation was resistant to L-NNA, *n*=3). Pretreatment with 10 μM indomethacin, a cyclooxygenase inhibitor, did not affect ACh-induced relaxation in the thoracic aortas of either group (data not shown). When the relaxant response to ACh was observed in high K^+ (45.4 mM) solution in the presence of L-NNA plus indomethacin, ACh did not cause relaxation.

SNP caused a dose-dependent relaxation of the aorta, which was significantly inhibited in the AHL compared with the ANL group (Table 3, Fig. 5). However, inhibition of SNP-induced relaxation in AHL aortas was far less than inhibition of ACh-induced relaxation. The maximum relaxation by SNP was not significantly different between the AHL and the ANL groups, while pD_2 for SNP in the AHL aortas was significantly decreased compared with that in the ANL aortas (Table 3). On the other hand, forskolin (30 nM–1 μM), a cyclic AMP generating agent, caused a dose-dependent relaxation of the aorta. The maximum relaxation and pD_2 in the AHL group (88.3± 2.7% and 7.05±0.14, *n*=6, respectively) did not differ from those in the ANL group (93.7±1.9% and 6.89±0.15, *n*=4, respectively).

3.3.2. Pulmonary arteries

The concentration of 5-HT required to induce 60% of the maximum contraction in the pulmonary arteries, with endothelium intact, in ANL and AHL mice was 0.25±0.06 μM (*n*=14) and 0.14±0.03 μM (*n*=11), respectively. This concentration of 5-HT was not statistically different between the AHL and ANL groups. Contraction induced by these concentrations were 72.7±7.8 mg (n=14) or 80.9±11.1 mg (n=11) for pulmonary arteries from ANL or AHL mice, respectively, and there was no significant difference in the level of pre-contraction in both groups. ACh-induced relaxation of AHL rings precontracted with 5-HT was

significantly inhibited compared with that of ANL rings (Fig. 1). The pD_2 value of ACh was 6.80±0.11 (n=11) and 7.16±0.12 (n=14, P<0.05) in the AHL and the ANL groups, respectively. The maximum relaxation induced by ACh was inhibited in AHL arteries (57.5±5.3%, n=11) compared with that in ANL arteries (89.6±2.0%, n=14, P<0.05, Fig. 1 and Table 3). ACh-induced relaxation was abolished by endothelial denudation (data not shown).

Pretreatment with L-NNA (200 μM) for 15 min completely inhibited ACh-induced relaxation of ANL and AHL pulmonary arteries (Table 3). Pretreatment with 10 μM indomethacin did not affect ACh-induced relaxation in pulmonary arteries from either group. SNP-induced relaxation of pulmonary arteries did not differ significantly between the AHL and ANL groups (Table 3).

3.4. Histology

On histological examination, no atherosclerotic change was observed in the thoracic aorta (Fig. 6) and pulmonary artery (data not shown) from AHL and ANL mice. Neither macrophages nor foam cells were observed.

4. Discussion

This is the first report to examine the endothelial function of arteries from European wood mice, a new model of hypercholesterolemia. Animals with total plasma cholesterol level >200 mg/dl formed the AHL (hypercholesterolemia) group and those with <200 mg/dl were the ANL (normocholesterolemia) group. Endothelium-dependent relaxation to ACh was impaired in arteries from AHL mice, although we did not detect any morphological change in these arteries. We found a negative

correlation between plasma total cholesterol and ACh-induced relaxation.

ACh releases different vasorelaxant mediators from the endothelium, e.g. NO (Ignarro *et al*., 1987), prostaglandin I_2 (PGI_2; Moncada *et al*., 1976) and endothelium-derived hyperpolarizing factor (EDHF) (Chen *et al*., 1988; Feletou and Vanhoutte, 1988). Their relative importance is variable, depending on the type and size of blood vessels. ACh-induced relaxation was significantly decreased in the thoracic aortas and pulmonary arteries of AHL mice. ACh-induced relaxation of the pulmonary artery is thought to be completely dependent on NO, since the eNOS inhibitor L-NNA abolished the relaxation response. On the other hand, part of the ACh-induced relaxation of the thoracic aorta was resistant to L-NNA (200 μM–1 mM). The cyclooxygenase inhibitor indomethacin did not affect ACh-induced relaxation in both arteries. This excludes the possibility that PGI_2 partly mediates ACh-induced relaxation. L-NNA-resistant relaxation of aorta from ANL mice was abolished in high KCl solution, the condition that eliminates the hyperpolarizing effect of EDHF (Brandes *et al*., 1999; Huang *et al*., 2005). Hence, EDHF is a candidate mediator of the L-NNA-resistant relaxation in the ANL aortas. This L-NNA-resistant relaxation was not detected in AHL aortas. Therefore, it is suggested that hypercholesterolemia impairs endothelial NO- and possibly EDHF-dependent relaxation. Some papers have shown that endothelial NO-dependent relaxation to ACh is impaired in thoracic aortas of hypercholesterolemic mice (apoE-deficient mice; d'Uscio *et al*., 2001), LDL receptor-deficient mice (Rabelo *et al*., 2003), apoE- and LDL receptor-deficient mice (Jiang *et al*., 2000), whereas EDHF-mediated relaxation is unaffected in common carotid arteries and resistance arteries in apoE-deficient mice (d'Uscio *et al*., 2001; Wolfle and de Wit, 2005). Intactness of EDHF-dependent component in arteries from apoE-deficient mice is contrast to the present result. This suggests that a change in

endothelial function in arteries from AHL mice is different from that of apoE-deficient mice.

Relaxation response to SNP, a nitrous compound that causes endothelium-independent relaxation, but utilizes the same second messenger cyclic GMP as endothelium-derived NO, was similar in AHL and ANL pulmonary arteries. However in the thoracic aortas of AHL mice, the sensitivity to SNP (pD_2) was slightly decreased without a change in the maximum response to SNP. On the other hand, the relaxant response to forskolin, a cyclic AMP-generating agent, was not impaired in AHL aortas, excluding the possibility that smooth muscle relaxation was generally impaired. Decreased response to SNP suggests that the ability of guanylate cyclase to produce cyclic GMP in response to NO is decreased, or the ability of cyclic GMP-dependent protein kinase to relax smooth muscle is decreased in AHL aortas. Since repression of the response to ACh was far greater than that to SNP, inhibition of NO generation in response to ACh is a major cause of the repressed response to ACh in AHL aortas. Taking these results together, it can be concluded that the endothelium-dependent relaxation of vascular smooth muscle is inhibited in arteries from AHL mice, mainly as a result of repression of NO generation and partly as a result of decreased sensitivity to NO, and possibly decreased activity of EDHF.

Total plasma cholesterol level in AHL mice was 219.5±4.8 mg/dl. As for other hypercholesterolemic models, total plasma cholesterol level has been reported to be >1000 mg/dl in SHL mice (Matsushima *et al*., 1999), >500 mg/dl in apoE-deficient mice (Plump *et al*., 1992; Ishibashi *et al*., 1993) and ~250 mg/dl in LDL receptor-deficient mice (Ishibashi *et al*., 1993). SHL mice or apoE-deficient mice spontaneously develop atherosclerosis when fed a normal diet (Matsushima *et al*., 1999). However, AHL and LDL receptor-deficient mice did not exhibit

atherosclerosis with a normal diet (Wulf *et al*., 1995). So far, we do not know whether a relatively higher level of plasma cholesterol determines the occurrence of atherosclerosis in SHL and apoE-deficient mice, or whether other factor(s) are responsible for initiation of atherosclerosis.

In spite of inhibition of endothelium-dependent relaxation, blood pressure and heart rate in AHL mice was not different from that in ANL mice. Intense inhibition of eNOS after long-term treatment with NOS inhibitor such as N^{ω}-nitro-L-arginine methyl ester causes hypertension in rats (Katoh *et al*., 1998; De Gennaro Colonna *et al*., 2005). Endothelial dysfunction in AHL mice is not as severe as that after long-term treatment with a NOS inhibitor, thereby it is possible that moderate inhibition of endothelial function does not affect the blood pressure in AHL mice.

Our data showed that plasma cholesterol level was not dependent on age in either AHL or ANL mice. These data are in agreement with those of a study showing that there is no correlation between cholesterol level and aging in *A. sylvaticus* mice (Shinohara *et al*., 2004). Interestingly, however, aging significantly reduced ACh-induced relaxation in the thoracic aorta of ANL mice. On the other hand, a correlation was found between hypercholesterolemia and inhibition of endothelium-dependent relaxation. It can be suggested from these data that hypercholesterolemia hastens aging of the endothelium. For the present, a causal relationship between hypercholesterolemia and endothelial dysfunction cannot be determined. In this respect, it would be interesting to know whether lowering plasma cholesterol by anti-cholesterol drugs (statin or fibrate) or low cholesterol diet improves endothelial function.

While total plasma cholesterol level in apoE-deficient or SHL mice is extremely high, that in AHL mice is moderately high and close to the level in most hypercholesterolemic patients. In this regard, AHL mice

could be a good model for diseases associated with hypercholesterolemia. In humans, hypercholesterolemia is not the sole factor to induce atherosclerosis, and an additional factor is necessary for its initiation and development. Therefore, using this model we may be able to find the factor(s) for initiation and development of atherosclerosis and clarify the relevance of hypercholesterolemia, endothelial dysfunction and the other key factor(s). Furthermore, these mice may be useful in the search to find a therapy for atherosclerosis.

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coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. Circulation 83, 391-401.

Table 1　Body weight and plasma total cholesterol level of ANL and AHL mice

	ANL			AHL		
	Young	Aged	All	Young	Aged	All
Age (weeks)	35.3±2.2	85.9±9.9	53.0± 6.6	39.5±7.5	96.5±6.3	87.8±8.0
Body weight (g)	25.8±1.2	25.7±2.1	25.7±1.0	27.4±8.4	28.6±1.6	28.4±1.6
Total cholesterol (mg/dl)	117.8±6.3	137.7±8.8	124.8±5.5	207.0±9.0*	221.8±5.3*	219.5±4.8*
n	13	7	20	4	11	15

Value are means±SEM.　*P<0.05 (vs. ANL).

Table 2　Blood pressure and heart rate in ANL and AHL mice

	ANL	AHL
Systolic blood pressure (mmHg)	121.2 ± 8.7	128.2 ± 7.5
Mean Blood pressure (mmHg)	80.1 ± 6.8	79.6 ± 2.4
Diastolic blood pressure (mmHg)	59.7 ± 7.3	55.3 ± 1.4
Heart rate	446.1 ± 23.6	498.6 ± 38.0
n	5	5

n, number of animals.

Table 3

Relaxation response to ACh and SNP in pulmonary arteries and thoracic aortas from ANL and AHL mice

Animals	Drugs	*n*	pD_2	Maximum relaxation
Thoracic aortas				
ANL	ACh	24	7.74±0.06	95.0±0.8 %
	ACh + L-NNA	24	-	23.9±3.6 % †
	SNP	28	8.47±0.06	93.7±1.2 %
AHL	ACh	25	7.20±0.12*	74.7±4.6 % *
	ACh + L-NNA	14	-	0.3±0.3 % †
	SNP	21	8.17±0.06*	91.5±1.3 %
Pulmonary arteries				
ANL	ACh	14	7.16±0.12	89.6±2.0 %
	ACh + L-NNA	14	-	0 % †
	SNP	5	8.13±0.12	98.0±1.1 %
AHL	ACh	11	6.80±0.11*	57.5±5.5 % *
	ACh + L-NNA	9	-	0 %†
	SNP	8	8.06±0.09	93.2±1.9 %

*P<0.05, vs. ANL aortas. †P<0.05, vs. ACh in the absence of L-NNA. Data on aortas were from 7-15 animals. Data on pulmonary arteries were from 5-10 animals.

Figure legends

Figure 1

Concentration-response curves for ACh-induced relaxation of thoracic aortas and pulmonary arteries from ANL (open circles) and AHL (closed circles) mice, in which the endothelium was preserved. Relaxation induced by papaverine (Pap) was taken as 100%. Each point represents mean±SEM of 14–39 preparations (7-15 animals). * Significantly different from ANL at the $P<0.01$ level.

Figure 2

Correlation between plasma total cholesterol level and ACh (1 μM)-induced maximum relaxation of thoracic aorta. Data are collected from the AHL and the ANL groups. Correlation coefficient ($r=0.73$) was significant ($P<0.0001$).

Figure 3

Concentration-response curves for ACh-induced relaxation of thoracic aortas from young (open squares) and aged (closed squares) mice, in which the endothelium was preserved. Relaxation induced by papaverine (Pap) was taken as 100%. Each point represents mean±SEM of 10–39 preparations (4-8 animals). *Significantly different from young aortas at the $P<0.01$ level.

Figure 4

Effects of L-NNA on ACh-induced relaxation in the endothelium-intact aortas from ANL and AHL mice. L-NNA (200 μM) was added 15 min before the arteries were precontracted with 5-HT. Open triangles, in the absence of L-NNA; closed triangles, in the presence of L-NNA. Each

point represents mean±SEM of 14–24 preparations (7-14 animals). *Significantly different from control at the $P<0.01$ level.

Figure 5

Concentration-response curves for SNP-induced relaxation of thoracic aortas from ANL (open circles) and AHL (closed circles) mice, in which the endothelium was preserved. Each point represents mean±SEM of 5–28 preparations. *Significantly different from ANL aortas at the $P<0.01$ level.

Figure 6

Micrographs of thoracic aortas from ANL and AHL mice stained with HE.

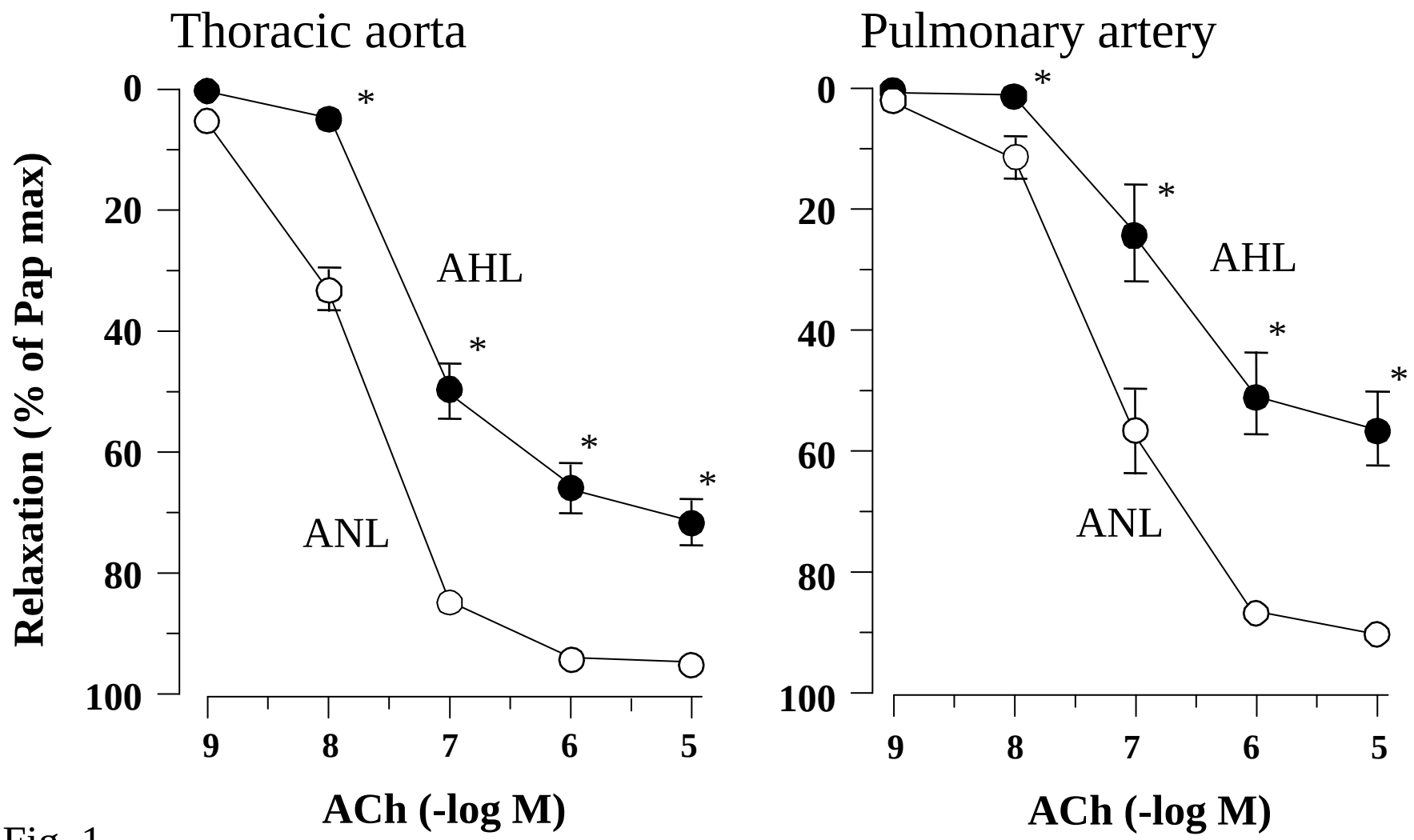

Thoracic aorta
Pulmonary artery
Relaxation (% of Pap max)
0
20
40
60
80
100
9
8
7
6
5
ACh (-log M)
AHL
ANL
*

Fig. 1

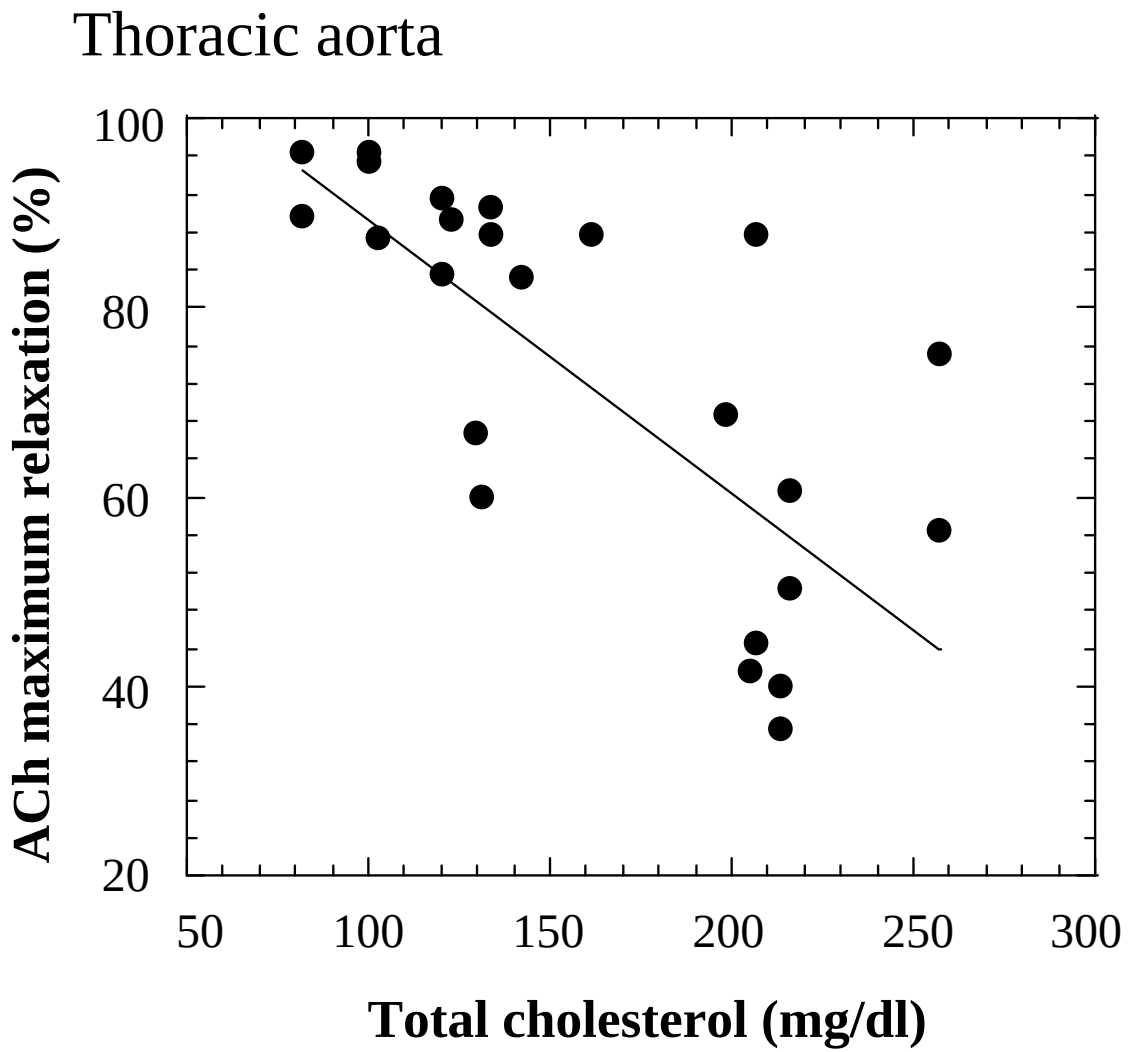

Thoracic aorta
ACh maximum relaxation (%)
100
80
60
40
20
50
100
150
200
250
300
Total cholesterol (mg/dl)

Fig. 2

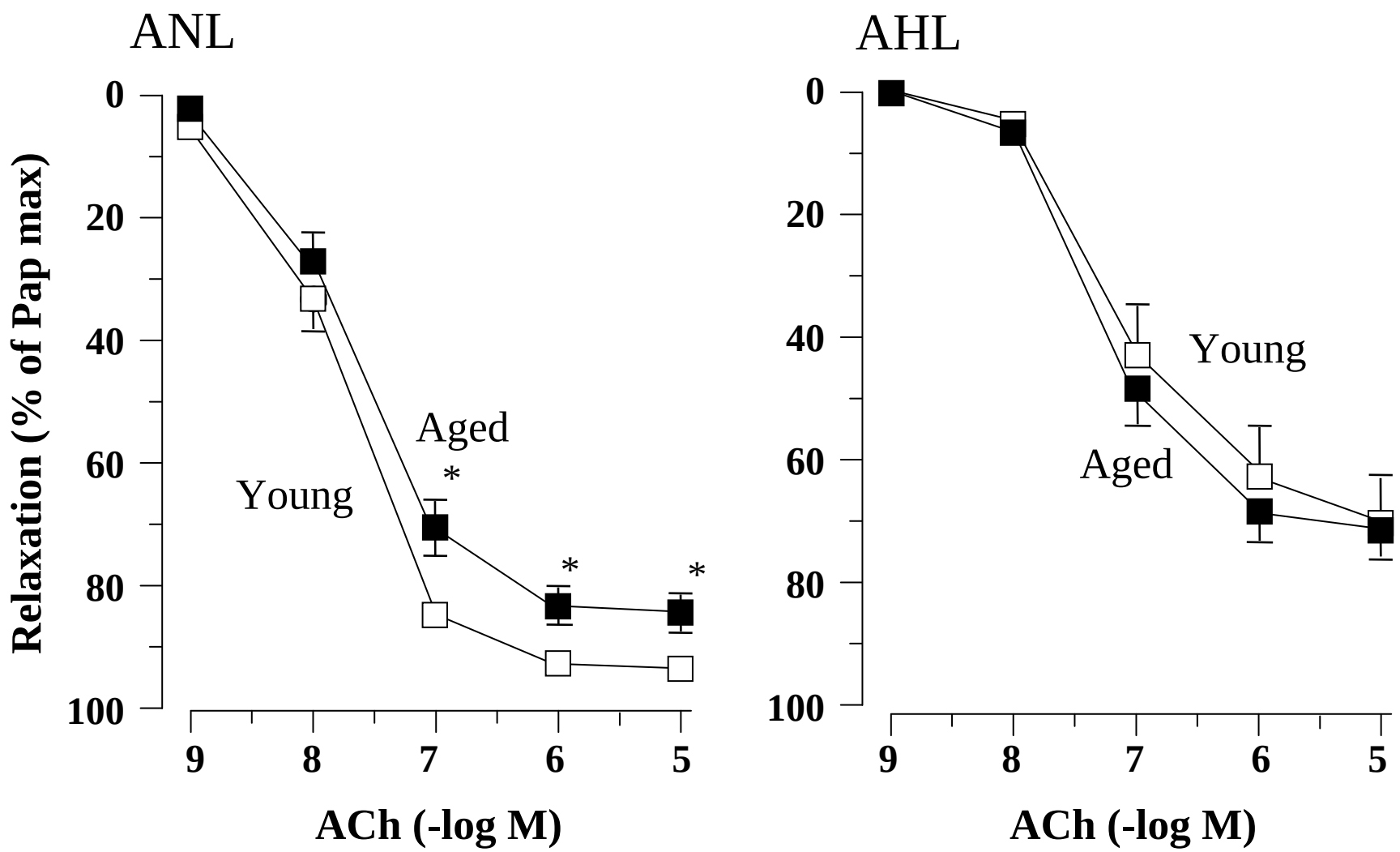

ANL
AHL
Relaxation (% of Pap max)
0
20
40
60
80
100
9
8
7
6
5
ACh (-log M)
Young
Aged
*
*
*

Fig. 3

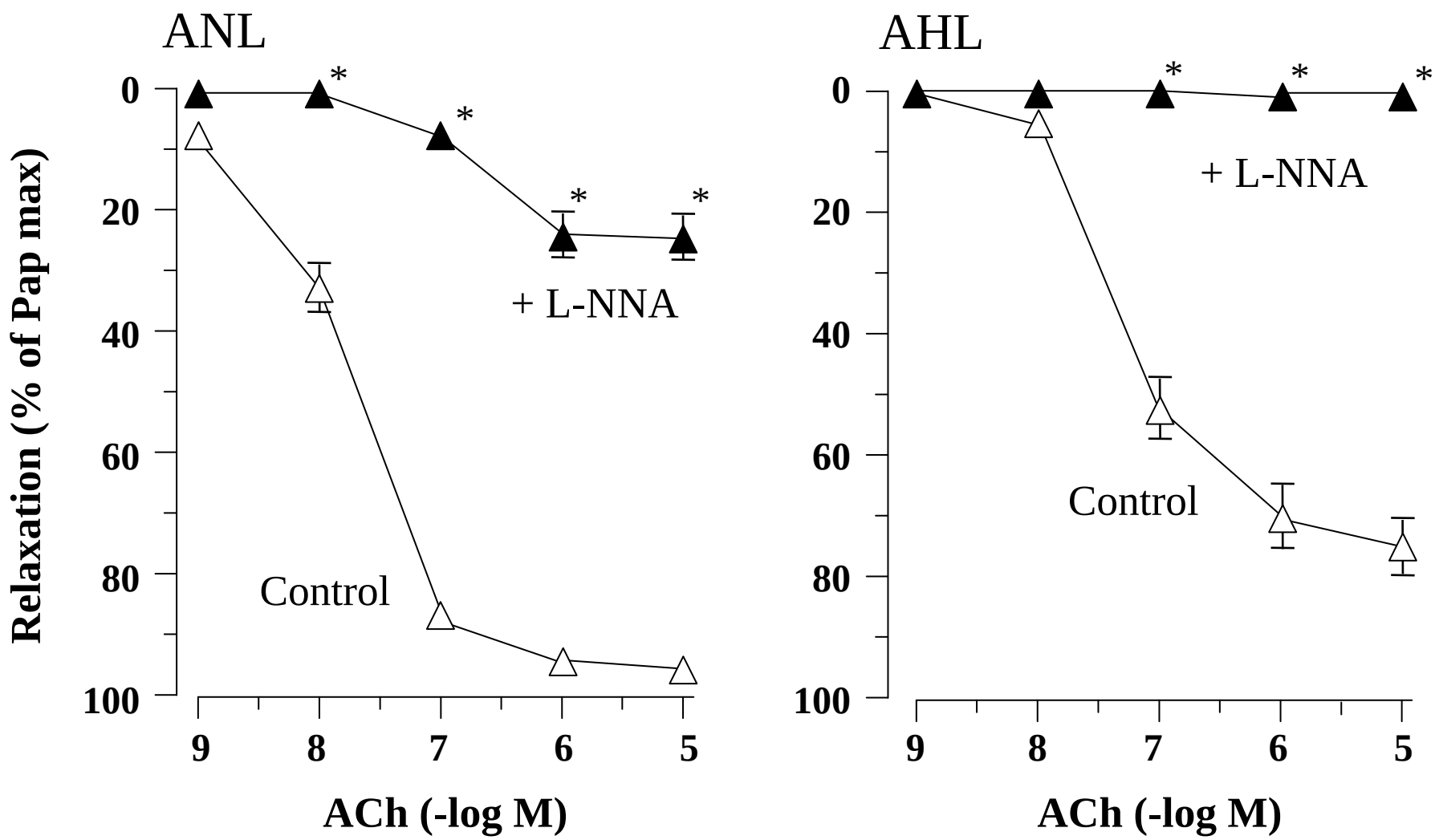

ANL
AHL
Relaxation (% of Pap max)
0
20
40
60
80
100
9
8
7
6
5
ACh (-log M)
+ L-NNA
Control
*

Fig. 4

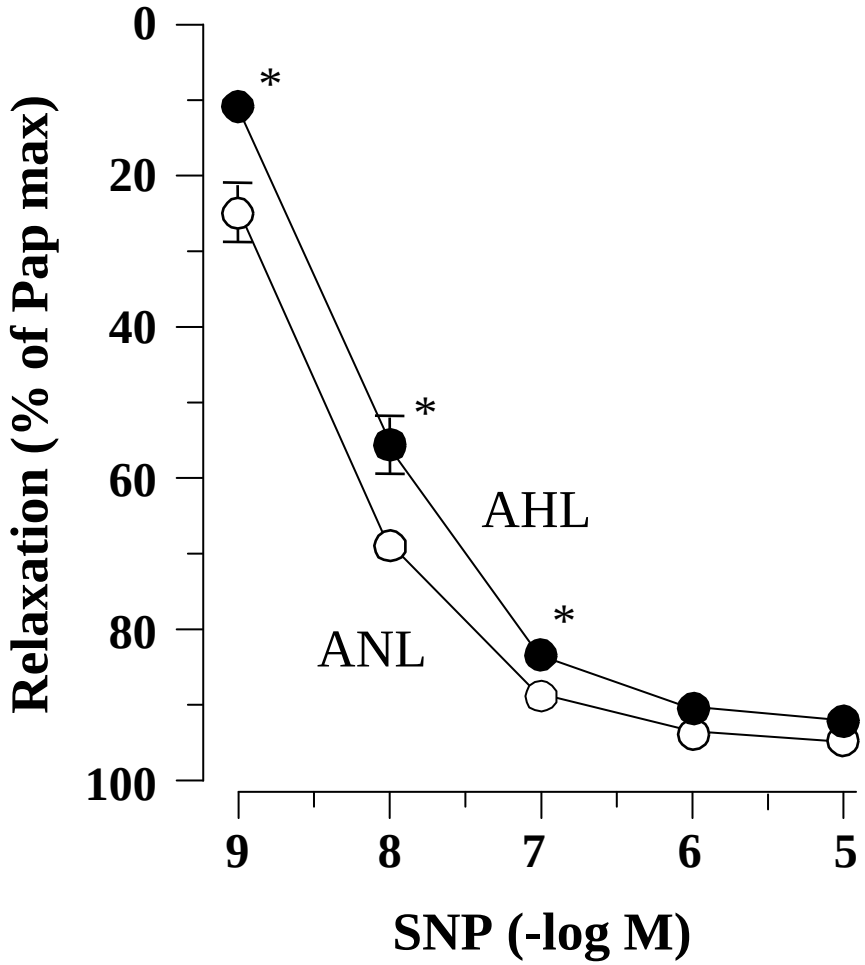
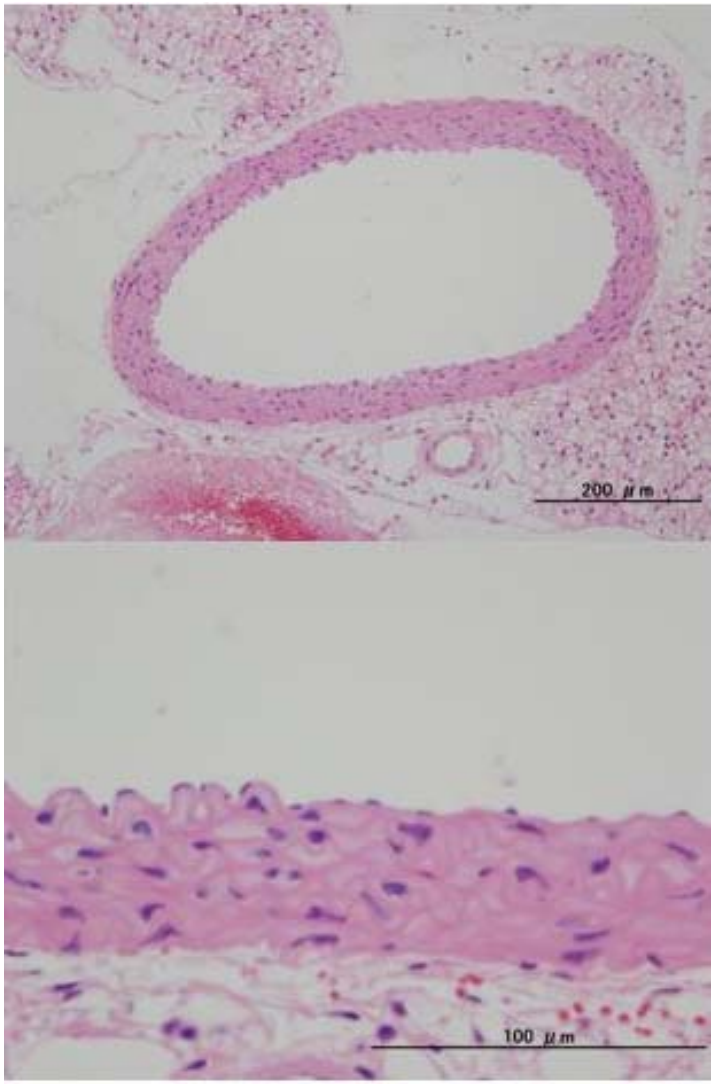


Fig. 5

ANL



AHL

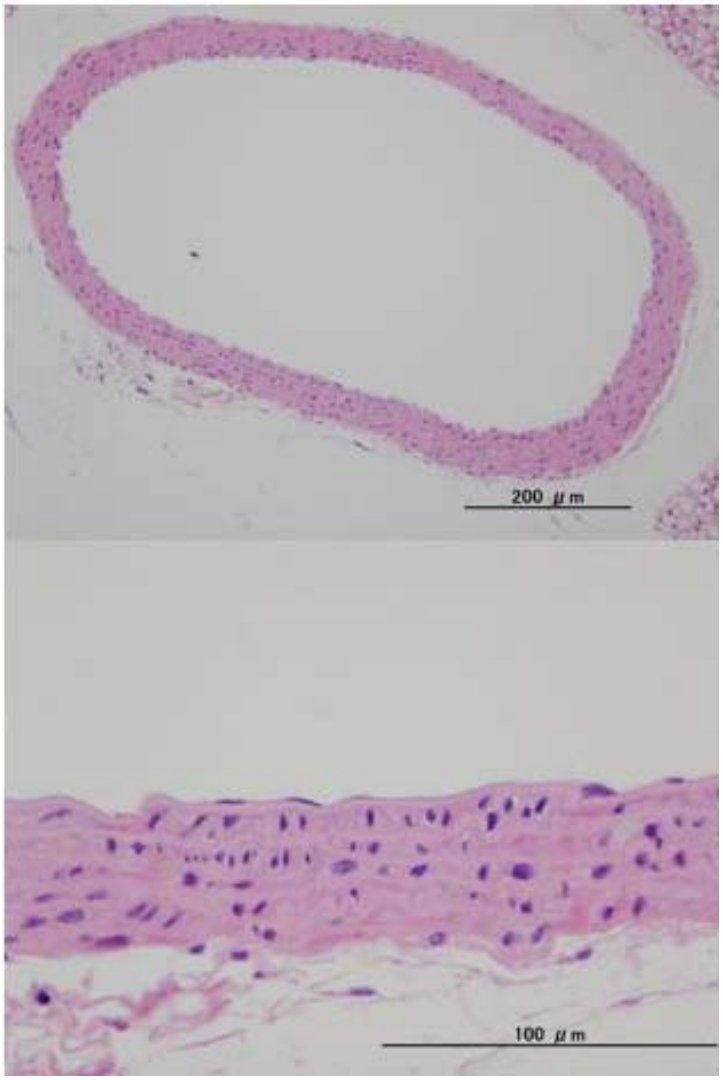


Fig. 6