

Age-Associated Endothelial Dysfunction in Rat Mesenteric Arteries: Roles of Calcium-Activated K^+ Channels (K_{ca})

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Summary

Age-associated changes in large blood vessels were characterized by increased arterial wall thickness, luminal dilation and impaired endothelial function. But little is known about the effect of age on structural and functional changes in small resistance arteries. The mechanisms underlying age-associated endothelial dysfunction in rat mesenteric resistance arteries were investigated in the present study. Small rat mesenteric arteries were excised and cannulated, and vascular endothelial functions were tested by acetylcholine (ACh). Our experiments showed (1) endothelium-dependent vasorelaxation induced by ACh was reduced in aged mesenteric arteries; (2) blockade of K_{ca} channels markedly reduced the vasodilation in young and adult rats, the resultant reduction in aged rats was much smaller compared with young and adult rats; (3) inhibition of endothelial nitric oxide synthase (NOS) resulted in a significant reduction of vasodilation in young and adult, but there was a smaller reduction in aged rats. The results suggest that (1) endothelial function was impaired in mesenteric arteries of aged rats; (2) both K_{ca} channels and nitric oxide (NO) contribute together to the ACh-induced vasorelaxation in small mesenteric arteries, and (3) both the impairment of K_{ca} channel function and decreased NO account for the age-related endothelial dysfunction.

Key words

Aging • Endothelial dysfunction • Potassium channels • Mesenteric artery • Acetylcholine

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Introduction

The process of aging is associated with marked changes in the vascular system that can lead to the development of cardiovascular diseases. It is known that increasing age induces structural and functional alterations in large conduit arteries (Santhanam *et al.* 2008) and small resistance arteries as well (Laurant *et al.* 2004, Briones *et al.* 2007). With age, the central aorta dilates (Lakatta 1993) and the arterial wall thickness of large arteries increases (Nagai *et al.* 1998). In small resistance arteries, age-related enlargement of vascular lumen and increased thickness of vascular wall have been reported recently (Briones *et al.* 2007, Laurant *et al.* 2004). Because age-associated cardiovascular disease is the leading cause of morbidity and mortality in industrialized and some developing countries, the mechanisms that initiate and propagate changes in the aging vasculature are currently in the focus of increasing investigation.

One of the major vascular functional changes with normal aging is the age-related endothelial dysfunction. Endothelial cells control the tone of the underlying vascular smooth muscle by releasing several constricting factors such as endothelin and prostaglandin H_2 , and endothelium-derived relaxing factors, including nitric

oxide (NO), reactive oxygen species (ROS), potassium ions (K^+), and metabolites of arachidonic acid. Age-related endothelial dysfunction may be due to an alteration of the balance between endothelium-derived relaxing and constricting factors (Matz *et al.* 2000). The reduced endothelium-dependent vasorelaxation with age has been characterized by reduced agonist-mediated vasodilation (Minamino and Komuro 2008), declined flow-induced vasodilation (Csiszar *et al.* 2002, Muller-Delp *et al.* 2002), and by decreased sensitivity of arteriolar endothelium to fluid shear stress (Sun *et al.* 2002). To date most studies have focused on the effects of aging on endothelium-dependent NO-mediated vasodilation. Endothelium-dependent vasorelaxation in aorta and large proximal arteries is dependent almost entirely on NO, and is very sensitive to increased endogenous superoxide (O_2^-) (Davidge *et al.* 1998, Knock *et al.* 2006). Moreover, in small resistance arteries, the effect of age on endothelium-dependent vasorelaxation has not been extensively studied (Matz *et al.* 2000). In distal resistance arteries, including those of mesenteric, pulmonary and cerebral circulation, however, endothelium-derived hyperpolarization factors (EDHF) participates significantly in agonist-induced vasorelaxation as the vessel diameter decreases (Sobey 2001). Hyperpolarization through potassium channel opening is a fundamental mechanism for vasorelaxation of small vessels. Calcium-activated K^+ channels (K_{ca}) are key effectors in the control of endothelium-dependent EDHF-evoked vasorelaxation (Hilgers and Webb 2007). There are three types of K_{ca} channels in vascular cells, including large-conductance K_{ca} (BK_{ca}) channels in vascular smooth muscle cells; intermediate (IK_{ca}) and small-conductance K_{ca} (SK_{ca}) channels in endothelial cells. Activation of K_{ca} channels on vascular cells contributes to agonist induced-vasorelaxation in rat (Dimitropoulou *et al.* 2007) and rabbit mesenteric arteries (Khan *et al.* 1993, Zhang *et al.* 2007). The endothelial cell IK_{ca} and SK_{ca} channels are especially important for EDHF-mediated relaxation and hyperpolarization in resistance arteries (Dora *et al.* 2008). Impaired K^+ channel function or reduced hyperpolarization in vascular cells has been detected in various diseased conditions such as hypertension (Hilgers and Webb 2007), diabetes (Fukao *et al.* 1997) and ischemia/reperfusion (Ko *et al.* 2008, Sobey 2001). But the role of K_{ca} channels during aging is still unclear. Given that small resistance arteries contribute to the regulation of blood pressure and local blood flow, EDHF and K_{ca} channels are implicated in control of blood pressure and regional blood flow in mammalian tissues *in vivo*, and may be altered under

pathophysiological conditions such as hypertension and diabetes as well as advancing aging. Changes in molecular composition and function of K_{ca} channels in resistance arteries may therefore be a fundamental event contributing to the progression of arterial dysfunction during age. However, EDHF and K_{ca} channel functional changes in healthy aging are not completely understood and require further studies. In the present study, we investigated the roles of K_{ca} channels underlying the age-related vascular functional changes, and provided evidences for impaired potassium channel function as well as reduced release of NO in the acetylcholine (ACh)-induced vasodilation in small mesenteric arteries of aged rats.

Materials and Methods

Vessel preparation

Male Sprague-Dawley rats aged 3-6 months (young), 10-12 months (adult), and more than 24 months (aged) were used in the present study. The animals were fed standard rat chow and had free access to tap water, and maintained in the Institute of Experimental Animals following the procedures approved by the China Central South University Advisory Committee for Animal Resources. Rats were anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally). The mesenteric vascular bed was excised and placed on a cooling plate containing cold ($0-4^\circ C$) 3-(N-morpholino) propanesulfonic acid (MOPS) buffered physiological saline solution (see below) containing 1 % bovine serum albumin. The second to fourth branches of the arteries (200-300 μm in diameter) were cut, cleaned of adherent connective tissue, transferred to an organ bath (2.5 ml volume) mounted on the stage of an inverted video microscope (Zeiss 100TV). Arterial segments were cannulated at both ends onto glass micropipettes and secured, and the lumen of the vessel was filled with MOPS-buffered solution containing 1 % albumin. The transmural pressure was set at 80 mm Hg and continuously monitored. Neither transluminal flow nor oxygenation was applied to the cannulated vessels. The internal diameter of the vessels was recorded by a computerized diameter tracking system (Diamtrak, Montech Pty Ltd., Australia).

Experimental procedures

After cannulation, the mesenteric arteries were superfused continuously with a MOPS-buffered physiological saline solution ($37.5^\circ C$, PH 7.3) of the

following composition (in mmol/l): 144 NaCl, 3 KCl, 2.5 CaCl₂, 1.4 MgSO₄, 2.0 pyruvate, 5.0 glucose, 0.02 ethylenediaminetetraacetic acid (EDTA), and 2.0 3-(N-morpholino) propanesulfonic acid (MOPS), 1.21 NaH₂PO₄. The extraluminal solution was warmed from room temperature to 37.5 °C. After 30-min equilibration, the vessels were exposed to 10 μmol/l phenylephrine (PE), which produced a near maximum contraction response in vessels from all age groups. After the contraction reached a steady state, increasing dose of acetylcholine (ACh) were applied to incubation bath to elicit vasorelaxation. The vessel internal diameter was recorded once the vessel reached a steady state.

For vasorelaxation mechanism study, the following inhibiting experiments were conducted: 10 μmol/l N^ω-monomethyl-L-arginine (L-NMMA; a NO synthase inhibitor); 1 μmol/l charybdotoxin (BK_{ca} and IK_{ca} channel inhibitor); 1 μmol/l apamin (SK_{ca} inhibitor). ACh-induced vessel diameter changes were recorded before and after 30-min incubation with these inhibitors. All chemicals were obtained from Sigma, St. Louis.

Data collection and statistical analysis

All data are presented as mean ± S.E.M. Six to ten vessels from at least 6 rats were used in each group. The vessel diameter changes were presented as percentages (%) of dilation of the precontracted vessels, calculated as follows: % of vasodilation = [(D_{agonist} - D_{base}) / (D_{max} - D_{base})] × 100, where D_{max} is the maximum diameter of the vessel at 80 mm Hg at room temperature before equilibration, D_{base} is the vessel diameter at steady contraction state induced by PE before the ACh stimulation, and D_{agonist} is the diameter of the vessel after ACh stimulation. With this method, the maximum dilation is represented as 100 %, and baseline diameter is 0 %. Comparisons were made with the use of paired Student's *t* test or ANOVA with a *post-hoc* Bonferroni test, as appropriate. The acceptable level of significant was defined as *P* < 0.05.

Results

Increased body weight in aged rats was observed. Body weight was greater (*P* < 0.01) in aged (586.4 ± 11.2 g, *n* = 8) than in adult (412.3 ± 8.6 g, *n* = 8) and in young (313.6 ± 4.8 g, *n* = 8) rats. The passive maximum internal diameters at 80 mm Hg of intravascular pressure were 245.67 ± 8.36 μm, 259.50 ± 8.49 μm and 266.33 ± 9.40 μm in young, adult and aged rats respectively. No

significant increase was observed in small mesenteric artery internal diameters (*P* > 0.05, *n* = 10 for all three groups) in our preparation.

Phenylephrine (PE) contracted the cannulated mesenteric resistance arteries in a concentration-dependent manner (data not shown). The contraction induced by 10 μmol/l PE was slightly increased in old rats. PE caused vessel contraction of 48.01 ± 2.89 % (127.83 ± 5.49 μm) in young, 50.65 ± 1.23 % (128.20 ± 5.81 μm) in adult, and 54.96 ± 3.70 % (172.17 ± 6.20 μm) in aged rats.

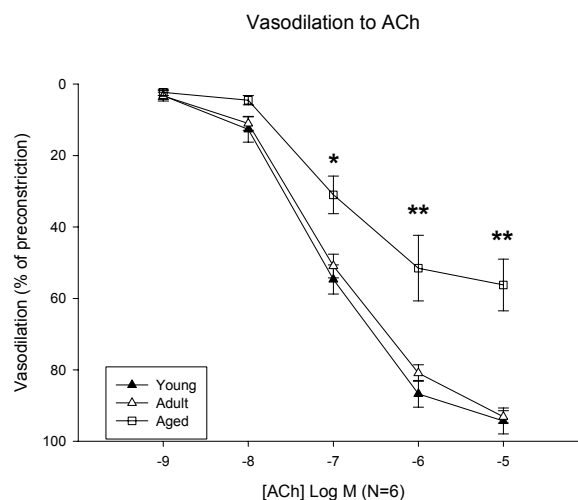


Fig. 1. Concentration-response curves to ACh in PE-precontracted small mesenteric arteries. ACh induced vasodilation of mesenteric arteries in a dose-dependent manner in young, adult and aged rats, but vasodilation was significantly declined in vessels from aged rats at the concentration 100 nmol/l, 1 μmol/l and 10 μmol/l. ** *P* < 0.001, * *P* < 0.05.

ACh-induced vasorelaxation in young, adult and aged rats are shown in Figure 1. Vascular relaxation responses to ACh were abolished by removal of endothelium (data not shown) in young rats, which supports that ACh-induced vasorelaxation in rat mesenteric arteries is endothelium-dependent. In young and adult groups, ACh induced similar vasorelaxation of rat mesenteric artery in a dose-dependent manner. There was no difference of ACh-induced dilation between young and adult groups. 1 and 10 μmol/l ACh evoked a vasodilation of 86.72 ± 1.54 % (229.83 ± 7.49 μm), 94.31 ± 3.47 % (238.83 ± 7.76 μm) in young, and 80.89 ± 0.94 % (234.43 ± 7.99 μm), 93.16 ± 1.71 % (250.50 ± 8.24 μm) in adult rats. However, the relaxation induced by ACh in the aged rats was significantly decreased at all ACh concentration except concentration of 1 and 10 nmol/l (Fig. 1, *P* < 0.001). 1 and 10 μmol/l ACh induced a

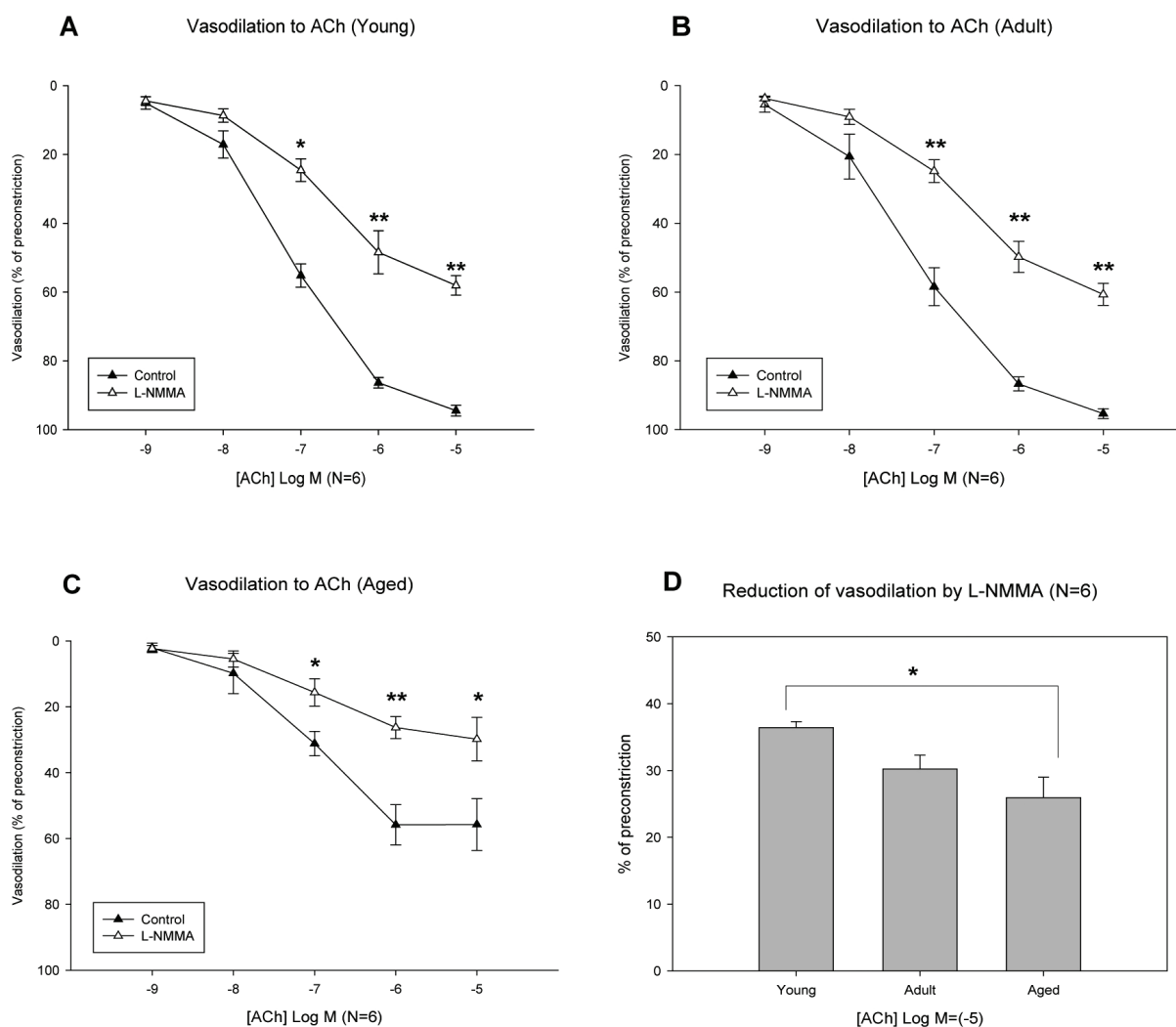


Fig. 2. Concentration-response curves to ACh before/after incubation with 10 $\mu\text{mol/l}$ L-NMMA in PE-precontracted small mesenteric arteries. L-NMMA incubation resulted in a significant reduction in vasodilation to ACh in young (A), adult (B) and aged rats (C) as well. But the reduction was declined in aged rats compared with young rats (D). ** $P < 0.001$, * $P < 0.05$.

dilation of 51.55 ± 3.75 % (222.83 ± 10.31 μm) and 56.25 ± 2.96 % (225.00 ± 9.20 μm). In young and adult rats, 10 $\mu\text{mol/l}$ ACh evoked almost fully dilation of mesenteric arteries. But in aged rat, 1 $\mu\text{mol/l}$ ACh induced the maximum vasodilation (less than 60 %) (Figs 1 and 2C), 10 $\mu\text{mol/l}$ (Fig. 1) and even 100 $\mu\text{mol/l}$ (data not shown) could not caused more vasorelaxation.

Nitric oxide is a well-known vasodilator in many vessel beds. PE-induced contractile responses were potentiated in all groups by L-NMMA (a specific NOS inhibitor). The contraction was 67.77 ± 2.96 % in young, 67.91 ± 2.32 % in adult, and 68.01 ± 2.02 % in aged rats after L-NMMA treatment. In young and adult rats, L-NMMA significantly reduced the vasorelaxation evoked by ACh (Figs 2A and 2B). After 30-min incubation of vessels with 10 $\mu\text{mol/l}$ L-NMMA, ACh-

evoked dilation was reduced from 86.37 ± 3.54 % (234.83 ± 4.87 μm), 94.46 ± 1.57 % (245.67 ± 5.73 μm) to 48.45 ± 2.56 % (164.17 ± 6.90 μm) and 58.07 ± 1.16 % (181.00 ± 4.37 μm) at 1 $\mu\text{mol/l}$ and 10 $\mu\text{mol/l}$, respectively. In adult rats, L-NMMA incubation evoked a similar inhibition (from 86.70 ± 2.03 % and 95.36 ± 1.42 % to 52.60 ± 2.18 % and 65.14 ± 2.25 %). In aged rats, L-NMMA also markedly inhibited the vasorelaxation induced by ACh (Fig. 2C). The vasodilation was reduced from 55.83 ± 2.50 % (222.50 ± 6.34 μm) and 55.76 ± 3.22 % (222.00 ± 7.02 μm) to 26.31 ± 1.37 % (186.83 ± 5.19 μm) and 29.83 ± 2.70 % (190.84 ± 6.35 μm) at ACh concentration of 1 $\mu\text{mol/l}$ and 10 $\mu\text{mol/l}$. Nevertheless, the reduction of vasodilation caused by incubation of L-NMMA was declined in aged rats (Fig. 2D) compared with young rats.

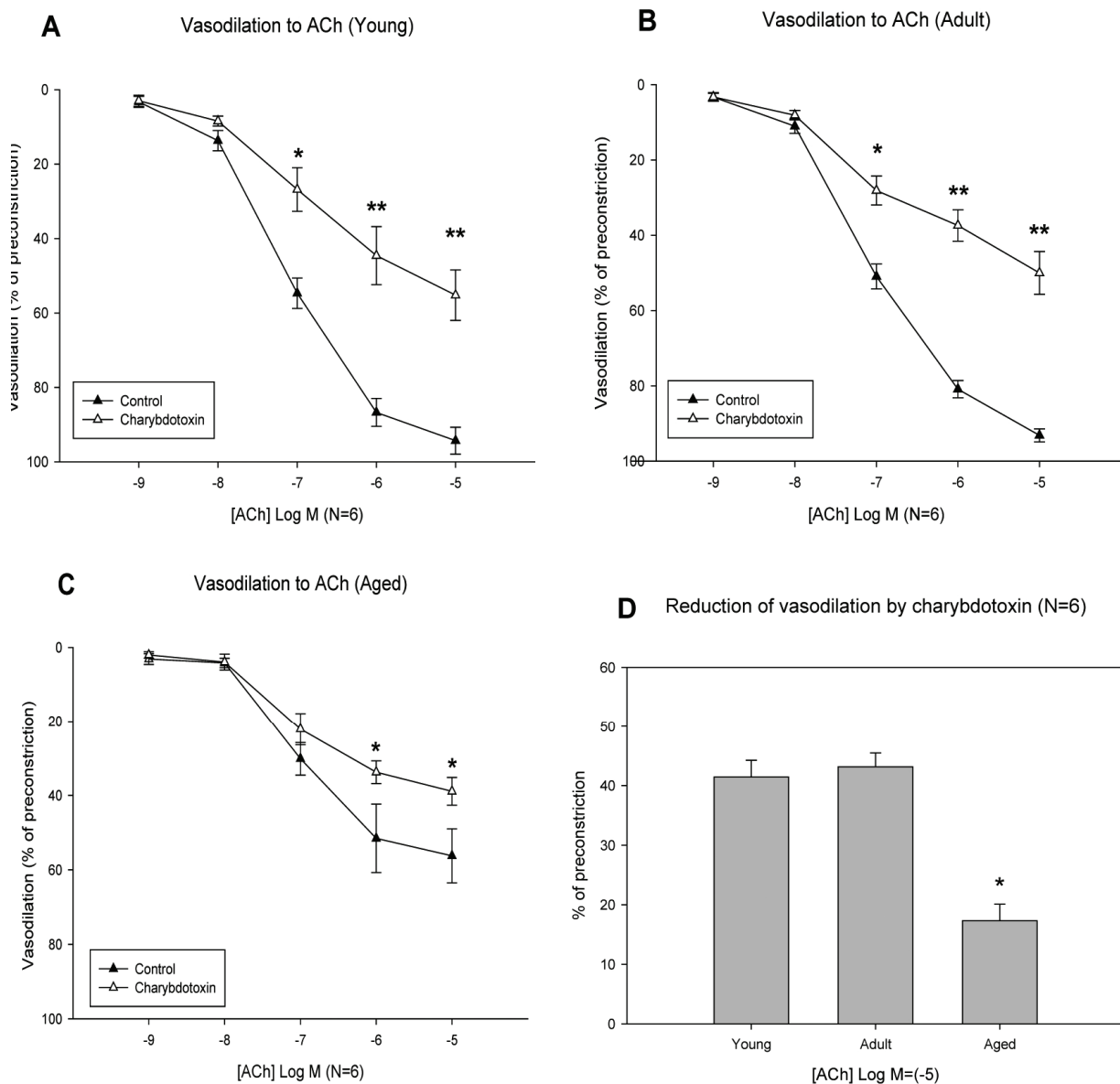


Fig. 3. Concentration-response curves to ACh before/after incubation with 1 $\mu\text{mol/l}$ charybdotoxin in PE-precontracted small mesenteric arteries. Incubation with charybdotoxin reduced the vasodilation to ACh in young (A), adult (B) and aged rats (C), but charybdotoxin resulted in a significantly lower reduction in aged rats compared with young and adult rats (D). ** $P < 0.001$, * $P < 0.05$.

Calcium-activated potassium channels (K_{ca}) play a central role in EDHF-mediated vasorelaxation in small mesenteric arteries (Hilgers and Webb 2007, Dora *et al.* 2008). In the present study, we used charybdotoxin to block IK_{ca} and BK_{ca} channels, apamin to block SK_{ca} channels. Incubation of vessels of young rats with charybdotoxin, ACh-induced vasorelaxation was reduced from 86.72 ± 3.76 % (230.83 ± 7.49 μm), 94.31 ± 3.61 % (238.84 ± 7.76 μm) to 44.59 ± 7.80 % (180.50 ± 5.57 μm) and 55.21 ± 6.77 % (192.83 ± 4.55 μm) at 1 and 10 $\mu\text{mol/l}$ ACh, respectively (Fig. 3A, $P < 0.001$). The reduced vasodilation was similar in adult rats (Fig. 3B). In aged rats, charybdotoxin also inhibited the ACh-induced

vasodilation (Fig. 3C). The vasodilation was reduced from 51.55 ± 3.75 % (220.86 ± 10.31 μm), 56.25 ± 3.91 % (225.00 ± 9.20 μm) to 33.78 ± 3.05 % (190.17 ± 8.83 μm) and 38.93 ± 1.52 % (196.16 ± 8.17 μm) at ACh concentration of 1 and 10 $\mu\text{mol/l}$. But the reduction was much less than that in young and adult rats (Fig. 3D). Incubation with apamin partially inhibited the ACh-induced vasorelaxation in young and adult rats (Figs 4A and 4B). Vasodilation in young rats was reduced from 86.72 ± 3.76 %, 94.31 ± 3.61 % to 60.56 ± 3.87 % (199.33 ± 6.55 μm) and 74.45 ± 6.54 % (216.00 ± 7.78 μm) at ACh concentration of 1 $\mu\text{mol/l}$ and 10 $\mu\text{mol/l}$, respectively (Fig. 4A, $P < 0.01$). In aged rats, incubation of

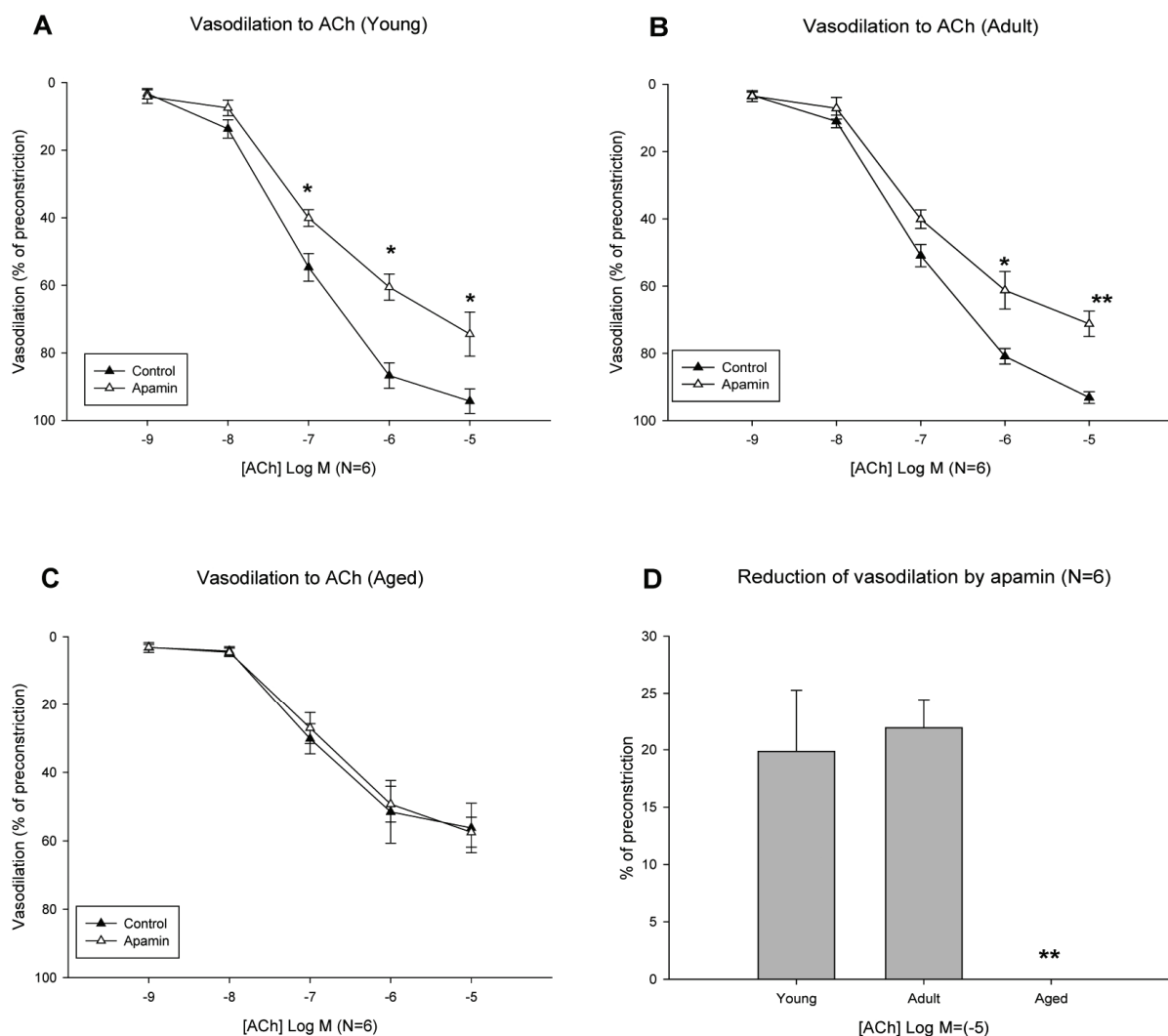


Fig. 4. Concentration-response curves to ACh before/after incubation with 1 $\mu\text{mol/l}$ apamin in PE-precontracted small mesenteric arteries. Apamin resulted in a significant reduction in vasodilation to ACh in young (A) and adult rats (B). Incubation of apamin did not have any effect on the ACh-induced vasodilation in aged rats (C). Comparison of the reduction of vasodilation by apamin was shown in Fig. 4D. ** $P < 0.001$, * $P < 0.05$.

vessels with apamin did not have significant effect on the dilation (Fig. 4C). ACh-evoked vasodilation was $49.30 \pm 5.21\%$ ($208.50 \pm 10.46 \mu\text{m}$) and $57.50 \pm 4.41\%$ ($218.00 \pm 9.31 \mu\text{m}$) after apamin treatment compared to $51.55 \pm 3.75\%$ and $56.25 \pm 2.96\%$ at 1 and 10 $\mu\text{mol/l}$ concentration. Figure 4D shows the reduction evoked by apamin at ACh concentration of 1 $\mu\text{mol/l}$. Apamin-induced reduction was declined with advancing age.

To further analyze the interactions of NO and K_{Ca} channels in aged rats, we tested the small mesenteric arteries with combined incubation of apamin, charybdotoxin and L-NMMA. Combined use of apamin, charybdotoxin and L-NMMA almost abolished the ACh-induced vasorelaxation in young rats (Fig. 5A). The

dilation was only $13.92 \pm 3.06\%$ at 1 $\mu\text{mol/l}$ ACh and $17.84 \pm 3.50\%$ at 10 $\mu\text{mol/l}$. Similar results were obtained from adult rats (Fig. 5B). In aged rats, vasodilation after combined application of apamin, charybdotoxin and L-NMMA was $10.83 \pm 2.74\%$ at 1 $\mu\text{mol/l}$ and $14.35 \pm 1.59\%$ at 10 $\mu\text{mol/l}$ (Fig. 5C). Vasodilation after combined incubation of apamin, charybdotoxin and L-NMMA in aged rat was similar to that in young and adult rats (Fig. 5D, $P > 0.05$).

Discussion

In this study, we provided evidence for age-related impairment of endothelial function in rat small

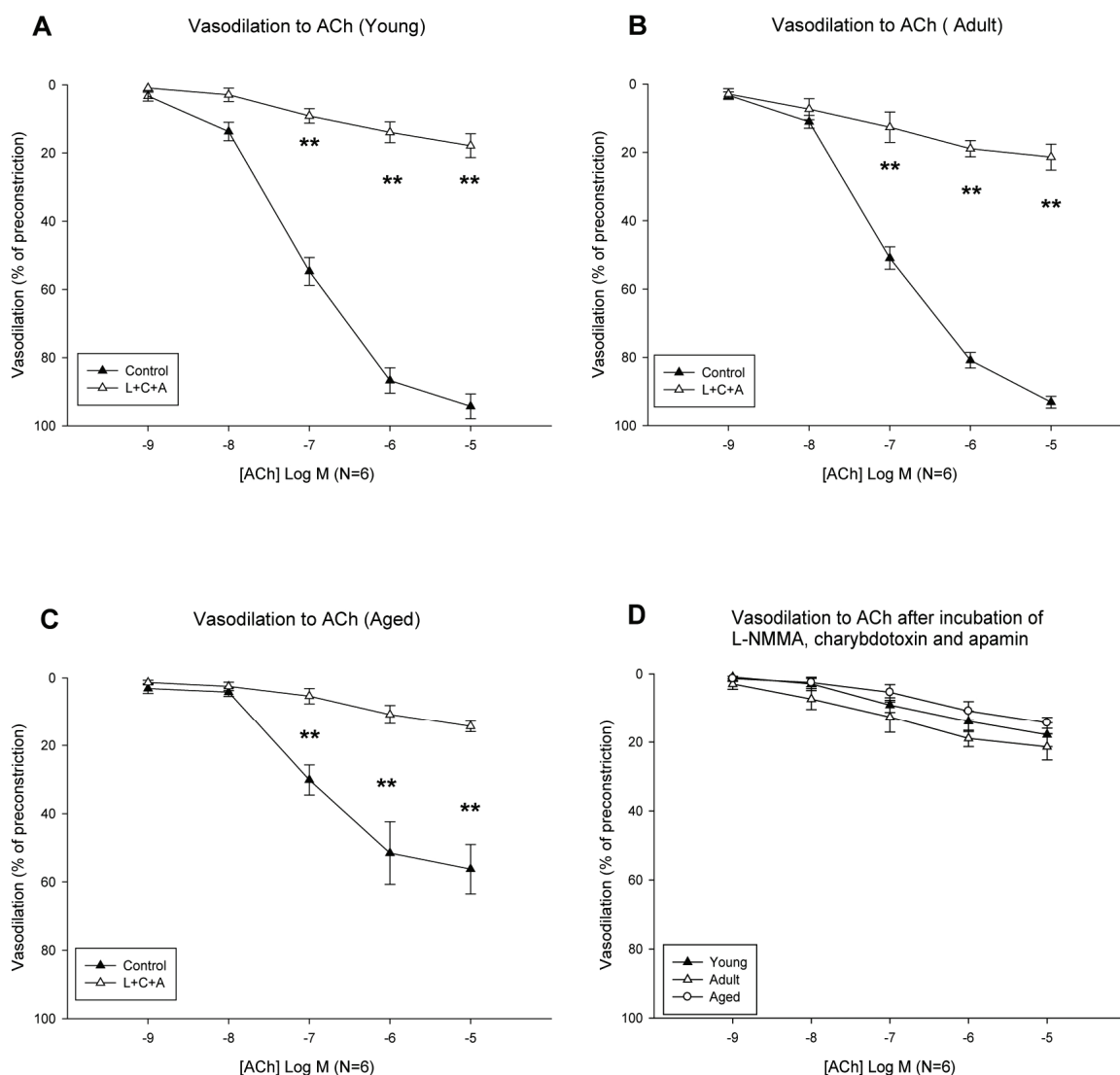


Fig. 5. Concentration-response curves to ACh before/after a combined incubation with 10 $\mu\text{mol/l}$ L-NMMA, 1 $\mu\text{mol/l}$ charybdotoxin and 1 $\mu\text{mol/l}$ apamin (L+C+A) in PE-precontracted small mesenteric arteries. The combined incubation almost abolished vasodilation induced by ACh in young (A), adult (B) and in aged rats (C). ACh-induced vasodilation was similar after combined incubation in three rat groups (D). ** $P < 0.001$.

mesenteric arteries. The major findings were (1) K_{Ca} channels and NO contribute to the dilation induced by ACh in rat small mesenteric arteries; (2) endothelium-dependent vasorelaxation was impaired in aged rats; (3) functionally defective K_{Ca} channels and reduced production of NO contribute to the age-associated impairment of endothelial function.

Vascular aging has been relatively well characterized in aorta and large proximal arteries. Age-associated changes in blood vessels include increased arterial wall thickness, luminal dilatation and impaired endothelial function (Minamino and Komuro 2008). However, little is known about the effects of age on small

resistance arteries. In the present study, we showed that the endothelium-dependent vasorelaxation to ACh in the small resistance mesenteric arteries declined with age. ACh-induced vasodilation was abolished by removal of endothelium, supporting the fact that ACh-induced vasorelaxation in rat mesenteric arteries is endothelium-dependent. In vessels from aged rats, ACh-induced vasorelaxation was markedly impaired compared to that in vessels from young and adult rats, indicating an age-associated impairment of endothelial function in small mesenteric arteries. In the present study, animal body weight increased with age, but we did not see an age-related increase in vascular internal diameter. In our

preparation, we chose vessels at almost the same size to fit our cannulation system, which may explain that there is no increase in vessel internal diameter.

Mechanisms accounting for the age-related endothelial dysfunction include an increase in oxidative stress, reduced endothelial NO synthase (eNOS) activity and NO production (Taddei *et al.* 2001, Sun *et al.* 2004, Minamino and Komuro 2008). The mechanism of increase in oxidative stress comprises increased production of reactive oxygen species (ROS), decreased NO bioavailability, and subsequent formation of peroxynitrite (ONOO⁻), which is well documented in aorta and large proximal arteries (Santhanam *et al.* 2008). In old rat aorta, endothelial NO synthase (eNOS) expression was increased, but NO production and downstream signaling (cGMP) was decreased (Cernadas *et al.* 1998). However, the mechanisms accounting for vasculature aging in distal resistance arteries may be different. Both the activity of eNOS and the release of NO have been suggested to be reduced in aged small resistance arteries (Amrani *et al.* 1996). eNOS mRNA and eNOS protein expression were reduced in the aging coronary (Csiszar *et al.* 2002) and skeletal muscle vasculatures (Woodman *et al.* 2002). In mesenteric arteries from aged rats, although eNOS protein expression was not different from that of young rats, shear stress-induced synthesis of NO was reduced (Sun *et al.* 2004). In our cannulated mesenteric arteries, inhibition of NOS significantly reduced the vasodilation induced by ACh in young, adult and aged rats, indicating that NO plays a predominant role in vasorelaxation of young, adult (Fujii *et al.* 1993) and aged vessels. Nevertheless, the inhibition of NOS resulted in a lower reduction in aged rats than that in young and adult rats, suggesting that NO production was decreased in ACh-induced vasodilation of aged rats. However, in the present study, age-related impairment of endothelial function was only partially attributed to the decreased NO production.

EDHF and K_{ca} channels have greater significance in agonist-induced vasorelaxation in small resistance arteries. Opening of potassium channels on vascular smooth muscle cells with resultant hyperpolarization plays a central role in the distal small resistance arteries. In cerebral penetrating arterioles, activation of BK_{ca} and IK_{ca} channels leads to arterial hyperpolarization, contributing to ATP-induced dilation (Dietrich *et al.* 2008). Genes encoding for SK_{ca} and IK_{ca} channel subunits were highly expressed in small

mesenteric arteries, stressing the importance of K_{ca} channels in these small arteries (Hilgers *et al.* 2006). In the present study, we used charybdotoxin to block BK_{ca} and IK_{ca} channels and apamin to block SK_{ca} channels. Both charybdotoxin and apamin significantly reduced the ACh-induced dilation in rat mesenteric arteries of young and adult rats, which provided evidences that K_{ca} channels contribute to the ACh-induced dilation in rat small mesenteric arteries.

Age-related structural alteration in small mesenteric arteries (Laurant *et al.* 2004, Briones *et al.* 2007) is likely accompanied by a decreased expression of K_{ca} channels, which was recently reported in small mesenteric arteries of hypertensive rats (Hilgers and Webb 2007). ROS, especially hydrogen peroxide (H₂O₂) effectively inhibited K_{ca} channel function (Brakemeier *et al.* 2003, Tang *et al.* 2004). Both enhanced ROS formation (Jacobson *et al.* 2007) and decreased expression of K_{ca} genes could lead to the impaired K_{ca} channel function in mesenteric arteries from aged rats, but the role of K_{ca} channels in mesenteric arteries of aged rats has not been documented before. In the present study, apamin incubation had no effect on the ACh-induced dilation in vessels from aged rats, suggesting that SK_{ca} channels are functionally defective in mesenteric arteries of aged rats. Blockade of IK_{ca} and BK_{ca} channels by charybdotoxin resulted in a significantly lower reduction of ACh-induced vasodilation in aged rats than in young and adult rats, suggesting that IK_{ca} and/or BK_{ca} channels are functionally impaired with age. A combined incubation of vessels with L-NMMA, charybdotoxin and apamin almost abolished ACh-induced vasodilation in young, adult and aged rats. There was no difference of the remaining dilation between these three animal groups. This further supported that K_{ca} channels and NO contribute to the ACh-induced vasorelaxation in small mesenteric arteries, and both impairment of K_{ca} channels and decreased NO production account for the age-related endothelial dysfunction.

In summary, in the present study, we showed that ACh-induced vasodilation was reduced in small resistance mesenteric arteries of aged rats; inhibition of K_{ca} channels and NOS markedly reduced the dilation in young, adult and aged rats, but resulted in a lower reduction in vessels from aged rats. Our results suggest that ACh-induced vasodilation was impaired in mesenteric arteries of aged rats, both K_{ca} channels and NO contribute to the vasodilation in these small resistance arteries, and the impairment of vascular

function in small mesenteric arteries of aged rats was attributed to impaired K_{ca} function and decreased NO production.

Conflict of Interest

There is no conflict of interest.

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