Age-associated endothelial dysfunction in rat mesenteric arteries : roles of calcium-activated K^+ channels (K_{ca})

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Running title: Age-associated endothelial dysfunction

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) blockage of K_{ca} channels markedly reduced the vasodilation in young and adult rats, the resultant reduction in aged rats was much less 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 a lower reduction in aged rats. The results suggest that (1) endothelial function was impaired in mesenteric arteries of aged rats; (2) K_{ca} channels and nitric oxide (NO) contribute together to the ACh-induced vasorelaxation in small mesenteric arteries, and (3) both 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 and acetylcholine

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 (Briones at al. 2007; Laurant et al. 2004). 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 at 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 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). But 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 , pumonary and cerebral circulation, however, endothelium-derived hyperpolarization factors (EDHF) is of increasingly greater significance in agonist induced vasorelaxation as the vessel diameter decreases (Sobey 2001). Hyperpolarization through potassium channel opening is a fundamental mechanism for vasorelaxation in 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 in EDHF-mediate 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 far from clear. 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 tissures 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 aged health are not completely understood and required 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 Sprage-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 ^{0}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 um 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 mmHg 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 ^{0}C, PH 7.3) of the following composition (in mmol/l): 144 NaCl, 3 KCl, 2.5 $CaCl_2$, 1.4 MgS O_4, 2.0 pyruvate, 5.0 glucose, 0.02 ethylenediaminetetraacetic acid (EDTA), and 2.0 3-(N-morpholino) propanesulfonic acid (MOPS), 1.21 NaH_2PO_4. The extraluminal solution was warmed from room temperature to 37.5 ^{0}C. After 30 minute 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 minute 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; The N represents the number of vessels. 6-10 vessels from at least 6 rats were used in a single group. The vessel diameter changes were presented as percentages (%) of dilation of the preconstricted vessels, calculated as follows: % of vasodilation=$[(D_{agonist}-D_{base})/(D_{max}-D_{base})]x100$, 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±11.2g, N=8) than in adult (412.3±8.6g, N=8) and in young (313.6±4.8g, N=8) rats. The passive maximum internal diameters at 80 mmHg 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.

ACh-induced vasorelaxation in young, adult and aged rats are shown in Fig 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. But 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 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 µmol/l ACh evoked almost fully dilation of mesenteric arteries. But in aged rat, 1 µmol/l ACh induced the maximum vasodilation (less than 60%) (Fig 1 and Fig 2C), 10 µmol/l (Fig 1) and even 100 µmol/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 (Fig 2A and 2B). After 30 minute incubation of vessels with 10 µmol/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 µmol/l and 10 µmol/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 µmol/l and 10 µmol/l. But the reduction of vasodilation caused by incubation of L-NMMA was declined in aged rats (Fig 2D) compared with young rats.

Calcium-activated potassium channels (K_{ca}) play a central role in EDHF-mediated vasorelaxation in small mesenteric arteries (Dora et al. 2008; Hilgers and Webb 2007). 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 μmol/l ACh respectively (Fig 3A, $P<0.001$). The reduced vasodilation was similar in adult rats (Fig 3B). In aged rat, 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 μmol/l. But the reduction was much less than that in young and adult rats (Fig 3D). Incubation of apamin partially inhibited the ACh-induced vasorelaxation in young and adult rats (Fig 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 μmol/l and 10 μmol/l respectively (Fig 4A, $P<0.01$). In aged rats, incubation of vessels with apamin did not have significant effect on the dilation (Fig 4C). ACh evoked vasodilation was 49.30±5.21% (208.50±10.46 μm) and 57.50± 4.41% (218.00±9.31 μm) after apamin treatment compared to 51.55±3.75% and 56.25±2.96% at 1 and 10 μmol/l concentration. Fig 4D showed the reduction evoked by apamin at ACh concentration of 1 μmol/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±3.06% at 1 μmol/l ACh and 17.84±3.50% at 10 μmol/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±2.74% at 1 μmol/l and 14.35±1.59% at 10 μmol/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 evidences for age-related impairment of endothelial function in rat small 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 were 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 that ACh-induced vasorelaxation in rat mesenteric arteries is endothelium dependent. In vessels from aged rats, ACh-induced vasorelaxation was markedly impaired than 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 (Minamino and Komuro 2008; Sun et al. 2004; Taddei et al. 2001). 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). But 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 aging 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 as well. But 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 are of increasing 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 in 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 (Briones et al. 2007; Laurant et al. 2004) 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 (H_2O_2) 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 did not have any 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 young and adult rats, suggesting that IK_{ca} /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 remained dilation between these three animal groups. It 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 evidences 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.

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Figure legends

Figure 1. Concentration-response curves to ACh in PE-preconstricted 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 100nmol/l, 1μml/l and 10 μmol/l. ** P<0.001, *P<0.05.

Fig 2. Concentration-response curves to ACh before/after incubation with 10 μmol/l L-NMMA in PE-preconstricted 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.

Fig 3. Concentration-response curves to ACh before/after incubation with 1 μmol/l Charybdotoxin in PE-preconstricted 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.

Fig 4. Concentration-response curves to ACh before/after incubation with 1 μmol/l apamin in PE-preconstricted 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.

Fig 5. Concentration-response curves to ACh before/after a combined incubation with 10 μmol/l L-NMMA, 1 μmol/l Charybdotoxin and 1 μmol/l apamin (L+C+A) in PE-preconstricted 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.

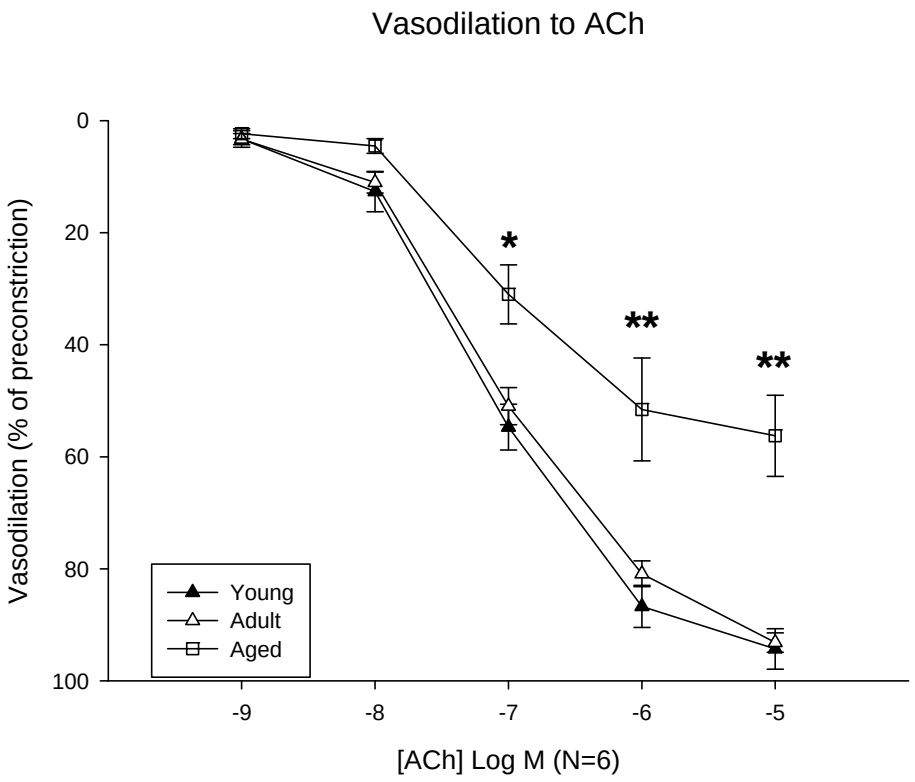
Vasodilation to ACh
Vasodilation (% of preconstriction)
0
20
40
60
80
100
-9
-8
-7
-6
-5
[ACh] Log M (N=6)
*
**
**
Young
Adult
Aged

Fig 1

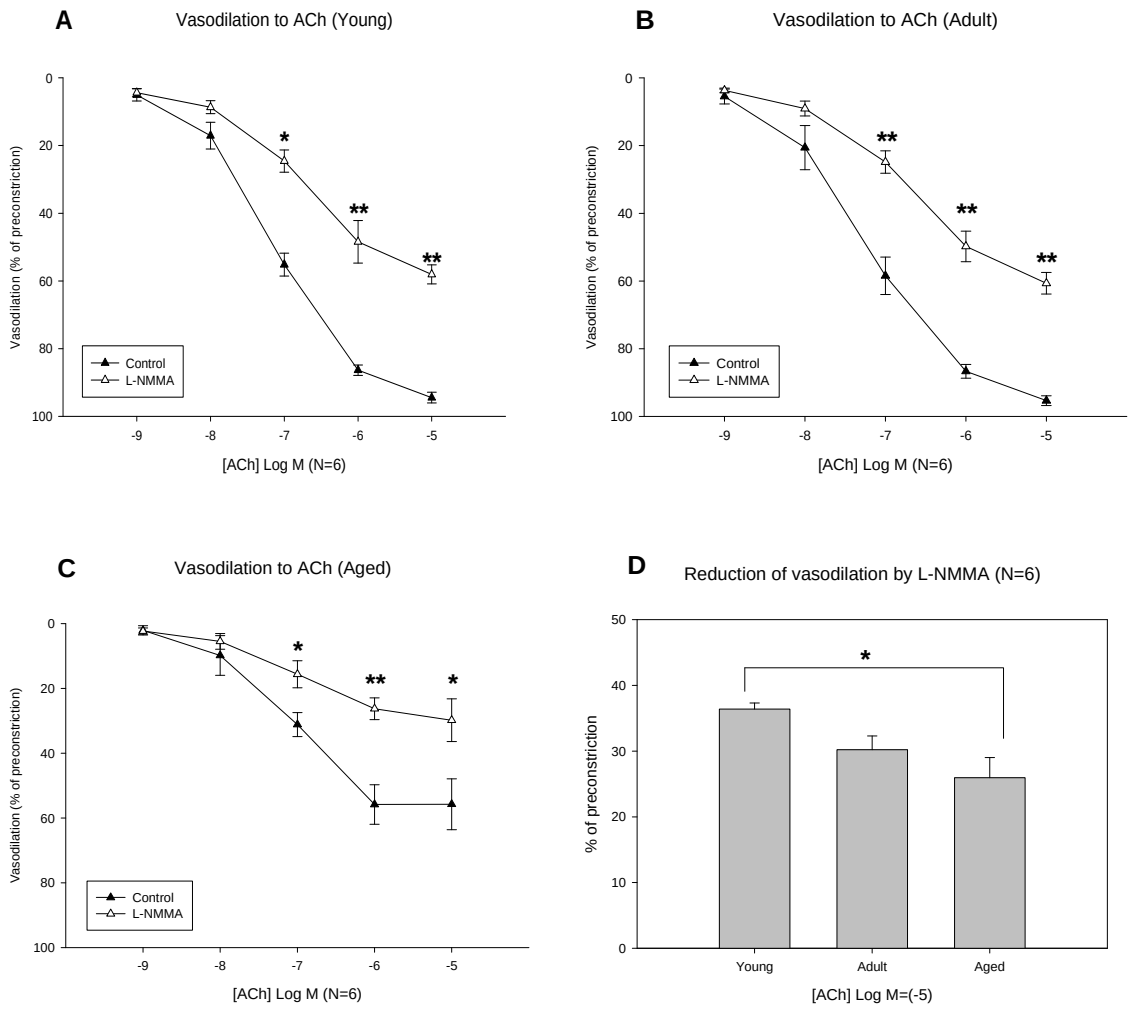
A
Vasodilation to ACh (Young)
Vasodilation (% of preconstriction)
[ACh] Log M (N=6)
Control
L-NMMA
B
Vasodilation to ACh (Adult)
Vasodilation (% of preconstriction)
[ACh] Log M (N=6)
Control
L-NMMA
C
Vasodilation to ACh (Aged)
Vasodilation (% of preconstriction)
[ACh] Log M (N=6)
Control
L-NMMA
D
Reduction of vasodilation by L-NMMA (N=6)
% of preconstriction
Young
Adult
Aged
[ACh] Log M=(-5)

Fig 2

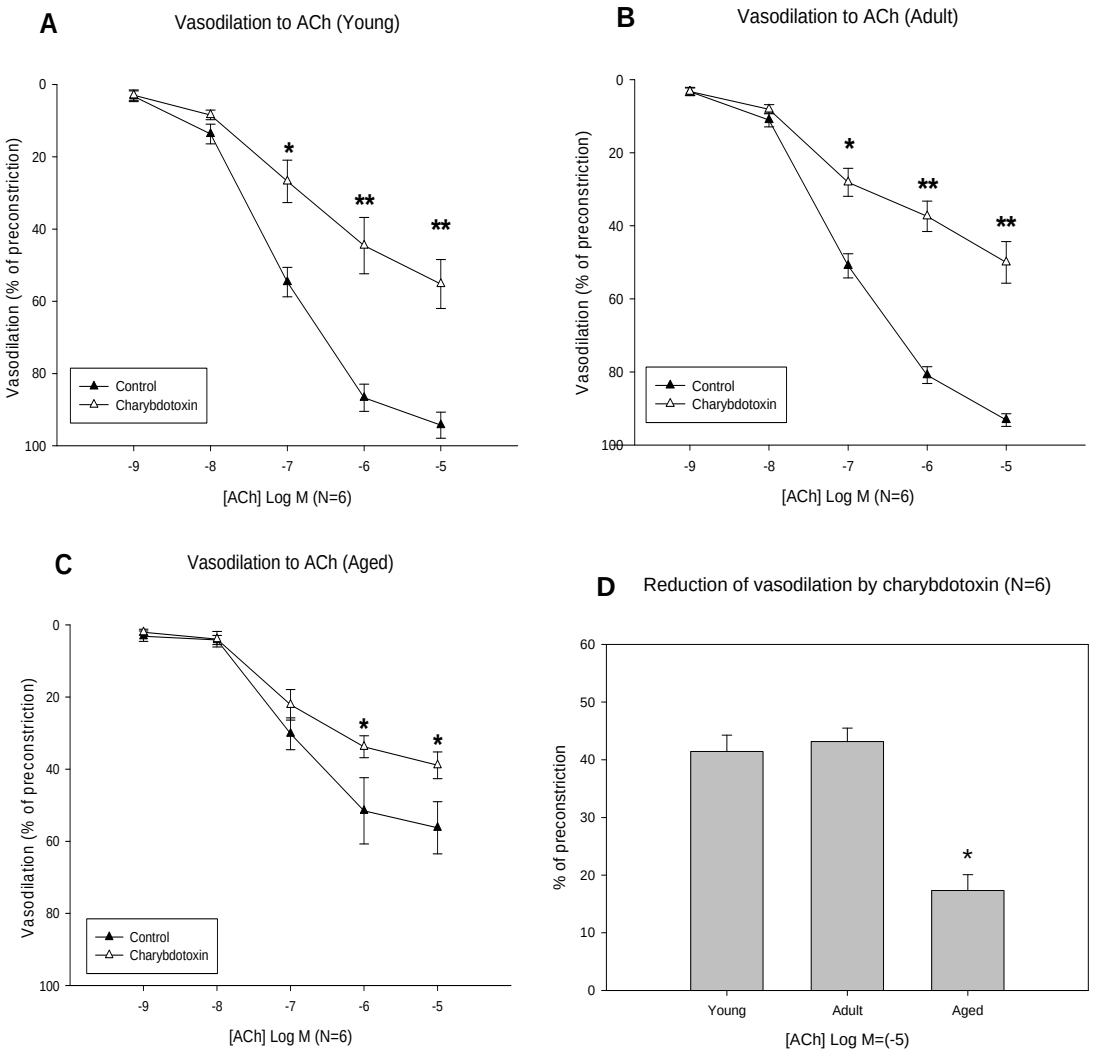
A
Vasodilation to ACh (Young)
Vasodilation (% of preconstriction)
[ACh] Log M (N=6)
Control
Charybdotoxin
B
Vasodilation to ACh (Adult)
C
Vasodilation to ACh (Aged)
D
Reduction of vasodilation by charybdotoxin (N=6)
% of preconstriction
Young
Adult
Aged
[ACh] Log M=(-5)

Fig 3

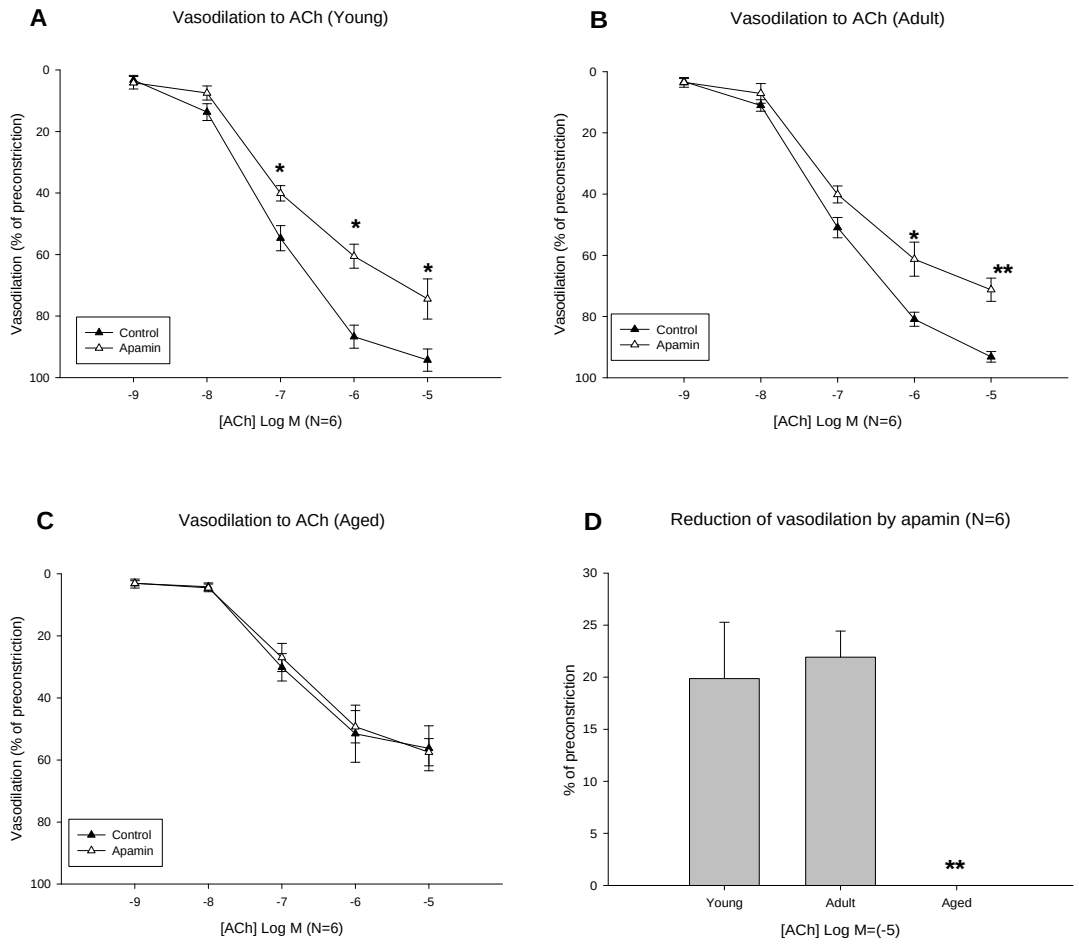
A
Vasodilation to ACh (Young)
Vasodilation (% of preconstriction)
0
20
40
60
80
100
-9
-8
-7
-6
-5
[ACh] Log M (N=6)
Control
Apamin
*
*
*
B
Vasodilation to ACh (Adult)
Vasodilation (% of preconstriction)
[ACh] Log M (N=6)
Control
Apamin
*
**
C
Vasodilation to ACh (Aged)
Vasodilation (% of preconstriction)
[ACh] Log M (N=6)
Control
Apamin
D
Reduction of vasodilation by apamin (N=6)
% of preconstriction
0
5
10
15
20
25
30
Young
Adult
Aged
**
[ACh] Log M=(-5)

Fig 4

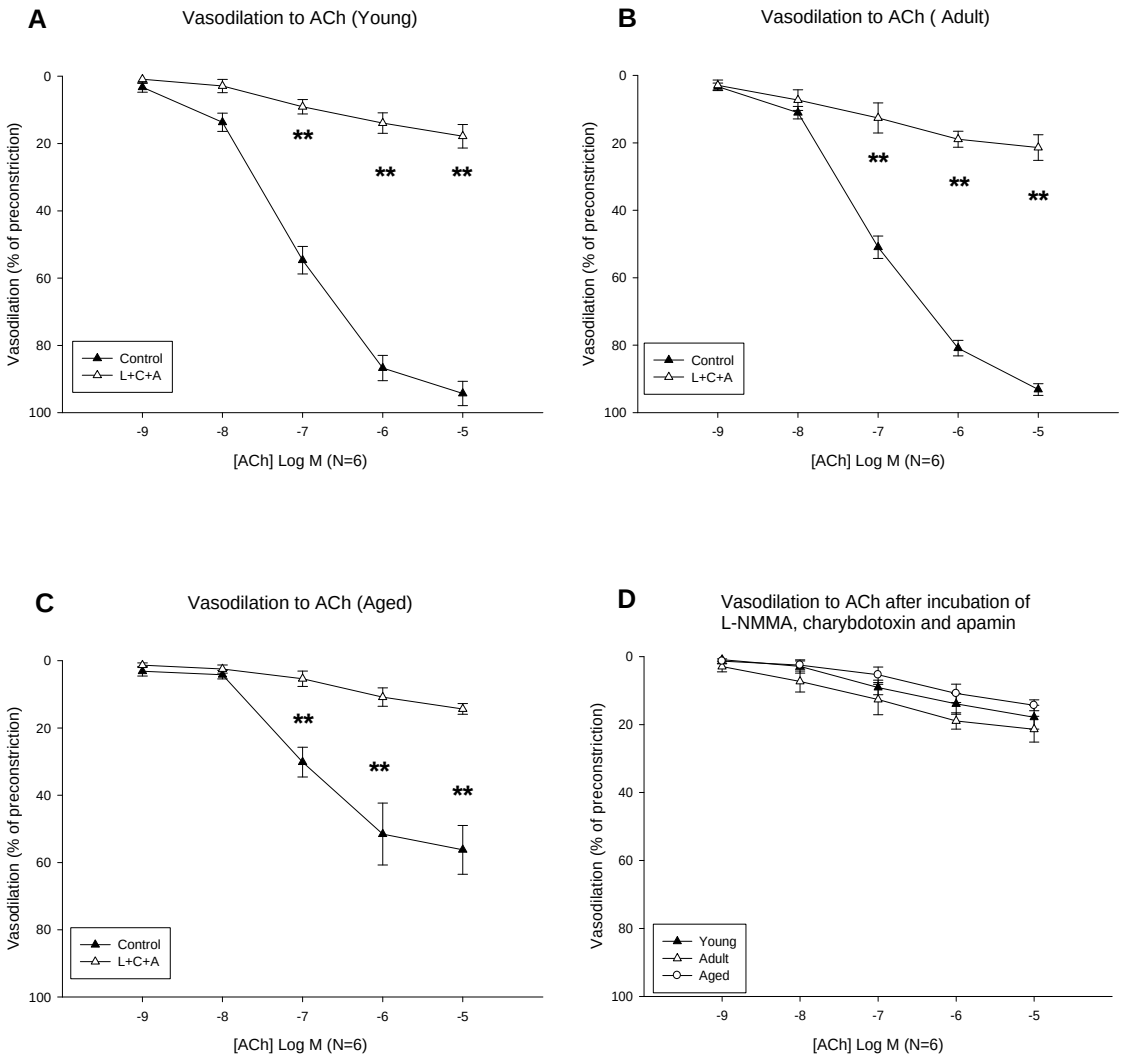
A
Vasodilation to ACh (Young)
Vasodilation (% of preconstriction)
[ACh] Log M (N=6)
Control
L+C+A
**
B
Vasodilation to ACh (Adult)
C
Vasodilation to ACh (Aged)
D
Vasodilation to ACh after incubation of
L-NMMA, charybdotoxin and apamin
Young
Adult
Aged

Fig 5