ELSEVIER

Cardiovascular Research 53 (2002) 487–495

Cardiovascular Research

www.elsevier.com/locate/cardiores

Chronic ET_A receptor blockade prevents endothelial dysfunction of small arteries in apolipoprotein E-deficient mice

Livius V. d'Uscio[a,1], Matthias Barton[c,1], Sidney Shaw[b], Thomas F. Lüscher[a,*]

[a]*Cardiovascular Research, Institute of Physiology, University of Zürich and Cardiology and University Hospital, Zürich, Switzerland*
[b]*Department of Clinical Research, University Hospital, Bern, Switzerland*
[c]*Clinical Atherosclerosis and Genetic Research Laboratory, University Hospital Zurich, Zurich, Switzerland*

Received 26 April 2001; accepted 28 August 2001

Abstract

Objective: This study investigated whether endothelial dysfunction occurs in mesenteric arteries of apoE-deficient mice and determined the role of endothelin (ET)-1, which is increased in human atherosclerosis, using an orally active endothelin ET_A receptor antagonist. **Methods:** ApoE-deficient and C57BL/6J control mice were fed for 30 weeks with normal chow or high-fat Western-type diet alone or in combination with darusentan (LU135252; 50 mg/kg/day). Vasomotor reactivity of isolated small mesenteric arteries (I.D. 200–250 μm) was studied in vitro under perfused and pressurized conditions. **Results:** In both mouse strains, about one fourth of the endothelium-dependent relaxant response to acetylcholine was insensitive to inhibition by L-NAME and indomethacin. In mesenteric arteries of apoE-deficient mice on Western-type diet, increased intima-media thickness and levels of endothelin-1 protein were observed. In addition, NO-mediated endothelium-dependent relaxation to acetylcholine was reduced without affecting L-NAME/indomethacin insensitive relaxation and contractions to endothelin-1 and serotonin were enhanced. Treatment with darusentan normalized vascular structure, NO-mediated relaxation to acetylcholine and contractions to endothelin-1 and serotonin without affecting blood pressure or plasma cholesterol levels. **Conclusions:** Severe hypercholesterolemia in apoE-deficient mice is associated with attenuation of NO-mediated relaxation to acetylcholine and increased vascular endothelin-1 content. Chronic ET_A receptor blockade may provide a new therapeutic approach to improve NO-mediated endothelium-dependent vasomotion in small arteries.

Keywords: Endothelins; Endothelial receptors; Endothelial function; Lipoproteins; Nitric oxide

1. Introduction

The vascular endothelium is a source of vasoactive substances such as nitric oxide (NO) [1] as well as endothelin-1 (ET-1) [2] that modulate vascular smooth muscle tone and proliferation. Endothelin (ET)-1 is a potent vasoconstrictor peptide [2] and its synthesis is stimulated by vasoactive factors implicated in atherogenesis [3]. ET-1 mediates contraction, cell proliferation [4,5], and vascular growth [6] via activation of ET_A-receptors. In hypercholesterolemia an elevated plasma ET-1 concentration and enhanced coronary artery tissue ET-1 immunoreactivity were found [7,8].

Nitric oxide (NO) is a potent vasodilator and is formed from L-arginine by endothelial NO synthase [1]. NO as well as prostacyclin inhibit ET-1 production [9] and furthermore, reduce ET-1 induced vasoconstriction in vascular smooth muscle cells of small arteries [10,11]. This indicates that NO protects small arteries against contractions to ET-1 under physiological conditions. In hypercholesterolemia, a major risk factor predisposing to atherosclerosis, endothelial dysfunction is present before structural vascular changes occurs [12]. In addition, in epicardial coronary arteries both atherosclerotic lesions and

*Corresponding author. University Hospital Zürich, Rämistrasse 100 CH-8091, Zürich, Switzerland. Tel.: +41-1-255-2121/2177; fax: +41-1-255-4251.

E-mail address: cardiotfl@gmx.ch (T.F. Lüscher).

[1]The first two authors contributed equally to this work.

Time for primary review 27 days.

0008-6363/02/$ – see front matter
PII: S0008-6363(01)00469-2

endothelial dysfunction are present while intramyocardial coronary arteries exhibit only impairment of endothelium-dependent vasodilation [13–16].

It is unknown whether endothelial dysfunction occur in mesenteric arteries of apoE-deficient mice, which develop spontaneous hypercholesterolemia and aortic atherosclerosis that can be accelerated by a lipid-rich, Western-type diet [17,18]. Using a selective, orally active antagonist for ET_A receptors, we investigated the role of ET-1 in small arteries in this mouse model of human atherosclerosis.

2. Methods

2.1. Animals and blood pressure

Male C57BL/6J (control) mice and homozygous apoE-deficient mice (C57BL/6J-ApoE^{Tm1Unc129}) were obtained at the age of 4–5 weeks from The Jackson Laboratory (Bar Harbor, ME, USA). Housing facilities and all experimental protocols were approved by the local authorities for animal research (Kommission für Tierversuche des Kantons Zürich, Switzerland). To accelerate the development of spontaneous atherosclerotic lesions [19], control and apoE-deficient mice were fed a Western-type fat diet for 30 weeks (0.15% cholesterol and 42% of milk fat by weight, TD88137, Harlan Teklad, Madison, WI, USA) with or without the ET_A receptor antagonist darusentan (50 mg/kg/day). In a separate protocol, apoE-deficient mice received normal chow diet with or without darusentan (50 mg/kg/day) for 30 weeks. Darusentan was mixed in powdered form to the diet and dosed as previously described [20,21]. Systolic blood pressure and heart rate were measured in conscious mice by a tail-cuff method using a custom-made pulse transducer (Model LE 5000, Letica, Barcelona, Spain).

2.2. Tissue harvesting

The mice were anesthetized (thiopental, 50 mg/kg body weight, i.p.) and sacrificed. A segment of the first branch of the mesenteric artery (closest to ileum; diameter: 200–250 μm) was isolated and placed in cold (4°C) modified Krebs–Ringer bicarbonate solution (in mmol/l: NaCl 118.6; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25.1; EDTA 0.026; glucose 10.1). Mesenteric arteries were dissected free from connective tissue under a microscope (Leica Wild M3C, Heerbrugg, Switzerland) and cut into rings of 4–5 mm in length.

2.3. Vascular function experimental set-up

Isolated mesenteric arteries were transferred to vessel chambers (Living Systems Instrumentation, Burlington, VT, USA), filled with Krebs–Ringer bicarbonate solution. The solutions circulating from a 250 ml reservoir at a flow-rate of 50 ml/min was aerated continuously with 95% O_2–5% CO_2 gas and kept at 37°C. The proximal and distal ends of the vessels were mounted and sutured onto two small glass microcannulas. The assembly was positioned in the vessel chamber and the perfusion pressure was set at 30 mmHg. In pilot studies, this was found to be optimal for contraction to norepinephrine as assessed by repeated exposures at various perfusions pressures (15–105 mmHg) using a transducer (Living Systems Instrumentation) connected to the afferent cannula. The intraluminal flow-rate was maintained at 0.2–0.4 ml/min. The perfusion chamber was positioned on the stage of an inverted microscope (Nikon, TSM-F, Japan) with a video camera (Burle Lancaster, PA, USA). The amplified image was transmitted to a monitor and a video dimension analyzer (V91, Living Systems Instrumentation), allowing for measurements and recording of lumen diameter and wall thickness.

2.4. Experimental protocols

Arteries were equilibrated for 60 min and perfused intraluminally with Krebs solution containing 1% BSA. Resting lumen diameter was recorded during perfusion with Krebs solution circulating from the reservoir. Between each protocol, the system was washed several times with Krebs solution and equilibrated for 30 min. Contractile responses to norepinephrine (10^{-10}–10^{-5} mol/l), serotonin (10^{-10}–10^{-5} mol/l) and ET-1 (10^{-11}–3×10^{-8} mol/l) were obtained. Endothelium-dependent relaxations to acetylcholine (10^{-9}–10^{-4} mol/l) after 30 min of preincubation with or without inhibitor of NO formation N^{ω}-nitro-L-arginine methyl ester (L-NAME; 10^{-4} mol/l) or indomathacin (10^{-5} mol/l) alone or in combination of both were obtained after stabilization of half-maximal precontraction of mesenteric arteries with norepinephrine. For endothelium-independent relaxations, sodium nitroprusside (10^{-10}–3×10^{-5} mol/l) was used.

2.5. Plasma cholesterol levels

Blood samples were obtained through puncture of the right ventricle. Blood was immediately transferred to a tube containing EDTA and centrifuged at 4°C for 10 min. Plasma was separated immediately at 4°C and kept at −80°C until assayed. Plasma LDL and HDL cholesterol were determined using a colorimetric-based assay on a Cobas Mira Plus® autoanalyzer (Roche Diagnostics, Basel, Switzerland).

2.6. Vascular ET-1 Protein Levels

Remaining mesenteric arterial tissue (n=4–8 per group) was dissected free, blotted dry, frozen in liquid nitrogen and kept at −80°C until assayed. Frozen tissue was

crushed and homogenized as described [6]. Eluates were dried in a speed-vac and reconstituted in working assay buffer for the RIA. ET-1 was measured by RIA and the peptide was identified by reverse-phase HPLC and related to tissue weight [21].

2.7. Drugs

Acetylcholine hydrochloride, L-norepinephrine bitartrate, sodium nitroprusside dihydrate, 5-hydroxytryptamine (serotonin), indomethacin and L-NAME were from Sigma (St. Louis, MO, USA) and ET-1 was from Novabiochem (Läufelfingen, Switzerland). Darusentan (LU135252), the active (+)-isomer of LU127043, a non-peptide, selective ET_A receptor antagonist was a gift from Knoll, Ludwigshafen, Germany.

2.8. Data analysis

Sensitivity to different drugs was expressed as negative logarithm of the concentration causing half-maximal relaxation or contraction (pD_2 value). In addition, maximal contraction or relaxation (expressed as percentage of the decrease in the basal intraluminal diameter or of the increase in intraluminal diameter from the diameter obtained after precontraction, respectively) were determined for each individual concentration–response curve by non-linear regression analysis type sigmoid logistic using MATLAB® software as described elsewhere [20,22]. CSA was calculated as follows [23,24]: $(\pi/4)\cdot[(\text{I.D.}+2\cdot(\text{intima}+\text{media})]^2-(\text{I.D.})^2$. Results are given as mean±S.E.M. In all experiments, *n* equals the number of mice. For multiple comparisons, results were analyzed with ANOVA followed by Bonferroni's correction. Pearson's correlation coefficient was calculated by linear regression analysis. A P value <0.05 was considered significant.

3. Results

3.1. Mice characteristics

ApoE-deficient mice were normotensive and systolic blood pressure and heart rate did not differ between darusentan-treated apoE-deficient and control mice (Table 1). Body weight was increased in Western-diet fed apoE-deficient mice as compared with normal chow diet ($P<0.05$, Table 1) and was further increased in control mice on high-fat diet ($P<0.05$, Table 1). Heart weight was augmented in apoE-deficient mice on Western-type diet ($P<0.05$; Table 1). However, heart weight-to-body weight ratio was unchanged in apoE-deficient mice on Western-type diet as compared with normal chow diet (Table 1). Treatment with ET_A receptor antagonist had no effect on these parameters in all groups of treated mice (Table 1).

Plasma total cholesterol and LDL-cholesterol concentrations were elevated in apoE-deficient mice on normal diet as compared with C57BL/6J mice ($P<0.05$; Table 1). The Western-type diet further increased plasma cholesterol and LDL ($P<0.05$), but HDL was reduced ($P<0.05$; Table 1). Concomitant treatment with the ET_A receptor antagonist had no effect on plasma cholesterol and LDL in apoE-deficient mice (Table 1) but increased HDL cholesterol levels in C57BL/6J mice ($P<0.05$; Table 1).

3.2. Vascular structure

In mesenteric arteries, lumen diameter did not differ in apoE-deficient and C57BL/6J mice (Table 2). Wall thickness and CSA were slightly increased only in apoE-deficient mice on Western-type fat-diet ($P<0.05$; Table 2) and were prevented by concomitant treatment with darusentan ($P<0.05$).

3.3. Tissue ET-1 Levels

Tissue ET-1 content was increased in mesenteric arteries

Table 1
Characteristics of apoE-deficient and C57BL/6J control mice after 30 weeks treatment

Parameters	C57BL/6J	C57BL/6J+ darusentan	ApoE	ApoE+ darusentan	ApoE	ApoE+ darusentan
Diet	WC	WC	NC	NC	WC	WC
SBP (mmHg)	ND	127±2	124±2	130±2	128±2	127±1
Heart rate (beats/min)	ND	488±15	529±17	499±5	483±8	512±9
Body weight (g)	50±1	49±1	32±1	34±2	44±3*†	40±2*†
Heart weight (mg)	141±4	135±4	140±3	137±6	190±6*†	195±4*†
Heart/body weight (mg/g)	2.8±0.3	2.8±0.2	4.3±0.1*	4.1±0.3*	4.3±0.3*	4.9±0.2*
Total cholesterol (mmol/l)	3.6±0.4	5.9±0.3	7.6±0.5*	8.7±0.3*	18.8±2.6*†	16.2±0.6*†
LDL (mmol/l)	0.6±0.2	1.0±0.2	6.4±0.7*	6.9±0.8*	17.3±2.4*†	15.3±0.6*†
HDL (mmol/l)	2.7±0.2	4.8±0.3*	2.0±0.9	2.9±0.3	0.4±0.1*†	0.3±0.1*†

ApoE, apolipoprotein E deficient mice; C57BL/6J, control wild-type mice; darusentan, ET_A receptor antagonist; NC, normal chow diet; WC, Western-type chow diet; SBP, systolic blood pressure; ND, not determined. Data are means±S.E.M. of 5–9 mice.
*, $P<0.05$ vs. C57BL/6J control mice (ANOVA+Bonferroni).
†, $P<0.05$ vs. apoE-deficient mice on normal diet (ANOVA+Bonferroni).

Table 2
Morphological characteristics of mesenteric arteries from apoE-deficient and wild-type mice measured in perfused and pressurized conditions

Parameters	C57BL/6J	C57BL/6J+ darusentan	ApoE	ApoE+ darusentan	ApoE	ApoE+ darusentan
Diet	WC	WC	NC	NC	WC	WC
Lumen diameter (μm)	222±4	235±7	219±7	228±9	217±5	226±6
Intima+media thickness (μm)	18.2±0.2	17.4±0.5	17.7±0.3	17.0±1.2	20.9±0.7*†	16.8±0.4#
Intima+media CSA ($\times 10^3 \mu m^2$)	14.3±0.2	13.9±0.6	13.2±0.5	13.0±0.6	15.6±0.6*†	12.9±0.5#

ApoE, apolipoprotein E-deficient mice; C57BL/6J, control wild-type mice; darusentan, ET_A receptor antagonist; NC, normal chow diet; WC, Western-type chow diet; CSA, cross-sectional area. Data are means±S.E.M. of 6–10 mice.
*, $P<0.05$ vs. C57BL/6J mice
†, $P<0.05$ vs. apoE-deficient group on normal chow diet
#, $P<0.05$ vs. apoE-deficient group on western-type chow diet (ANOVA+Bonferroni).

of apoE-deficient mice on Western-type diet as compared with apoE-deficient mice on normal diet or C57BL/6J mice ($P<0.05$; Fig. 1). Darusentan prevented the increase in tissue ET-1 levels in apoE-deficient mice to a greater degree as compared with C57BL/6J mice on Western-type diet ($n=8$, $P<0.05$, Fig. 1). Darusentan did not significantly affect vascular ET-1 levels in apoE-deficient mice on normal diet ($n=4$).

3.4. Endothelial function

Acetylcholine-induced relaxation was endothelium-dependent. The relaxations were impaired in apoE-deficient mice as compared to control mice on Western-type diet ($P<0.05$ vs. C57BL/6J; Fig. 2A; $n=9$). In contrast, the relaxations were unaltered in apoE-deficient mice fed normal diet (Fig. 2B; $n=10$). Incubation with L-NAME alone reduced maximal responses to acetylcholine to a comparable degree in apoE-deficient and C57BL/6J mice

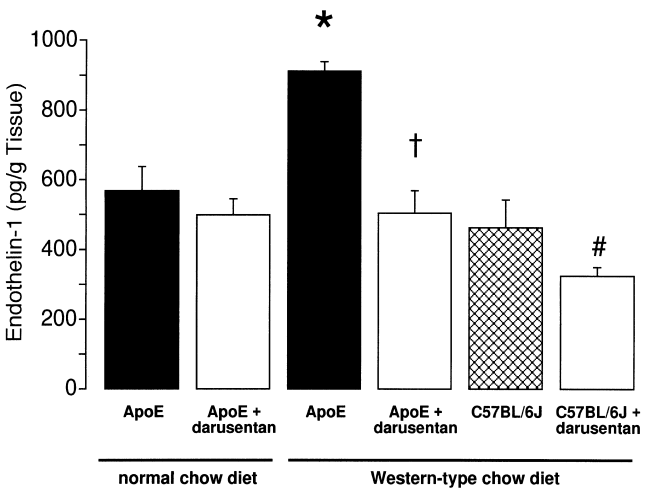

Fig. 1. Tissue concentrations of ET-1 in small mesenteric arteries of apoE-deficient and C57BL/6J mice after 30 weeks treatment with Western type diet alone or in combination with the ET_A receptor antagonist darusentan ($n=4$–8). For comparison, the results of normal fed apoE-deficient mice with or without darusentan are shown. *, $P<0.05$ vs. all other groups; †, $P<0.05$ vs. apoE-deficient mice on Western-type diet; #, $P<0.05$ vs. C57BL/6J mice (ANOVA+Bonferroni).

treated with and without darusentan ($P<0.05$ vs. without pretreatment; $n=5$–7; Fig. 2C and D). Preincubation with indomethacin in the presence or absence of L-NAME did not affect acetylcholine-induced relaxations (data not shown).

Chronic treatment with darusentan, increased the L-NAME-sensitive portion of the response in apoE-deficient mice on Western-type diet ($P<0.05$; $n=13$; Fig. 2A). Maximal responses were inversely correlated with tissue ET-1 level in apoE-deficient mice on Western-type diet ($r=-0.77$; $P<0.005$; $n=12$) but not in C57BL/6J mice ($P=0.95$; $n=13$; NS).

Sensitivity but not maximal responses of endothelium-independent relaxations to sodium nitroprusside were slightly lower in arteries from apoE-deficient mice as compared to controls ($P<0.05$ vs. C57BL/6J, Fig. 3A) and was increased after darusentan ($P<0.05$ vs. apoE-deficient mice; Fig. 3A; $n=5$–9), without affecting the maximal response. In C57BL/6J mice on Western-type diet or apoE-deficient mice fed normal diet, no differences within treatment groups were observed (Fig. 3A and B).

3.5. Vascular contractility

Maximal contractions to ET-1 were unaffected by hypercholesterolemia, but the sensitivity of contractions to ET-1 was slightly increased in apoE-deficient mice on Western-type diet as compared with control mice ($P<0.05$; $n=4$; Fig. 4); this shift was normalized by darusentan ($P<0.05$; $n=4$; Fig. 4A). Concomitant treatment with darusentan had no effect on contractions to ET-1 in C57BL/6J mice on western-type diet or in apoE-deficient mice on normal diet (Fig. 4B).

Maximal contractions to serotonin were markedly higher in mesenteric arteries of apoE-deficient mice as compared to controls fed Western-type diet ($P<0.05$; Fig. 5A). The supersensitivity to serotonin was completely blocked by chronic treatment with darusentan ($P<0.05$; $n=5$–7; Fig. 5A).

Contractions to norepinephrine were similar in apoE-deficient mice and C57BL/6J mice fed Western-type diet. Concomitant treatment with darusentan did not affect this

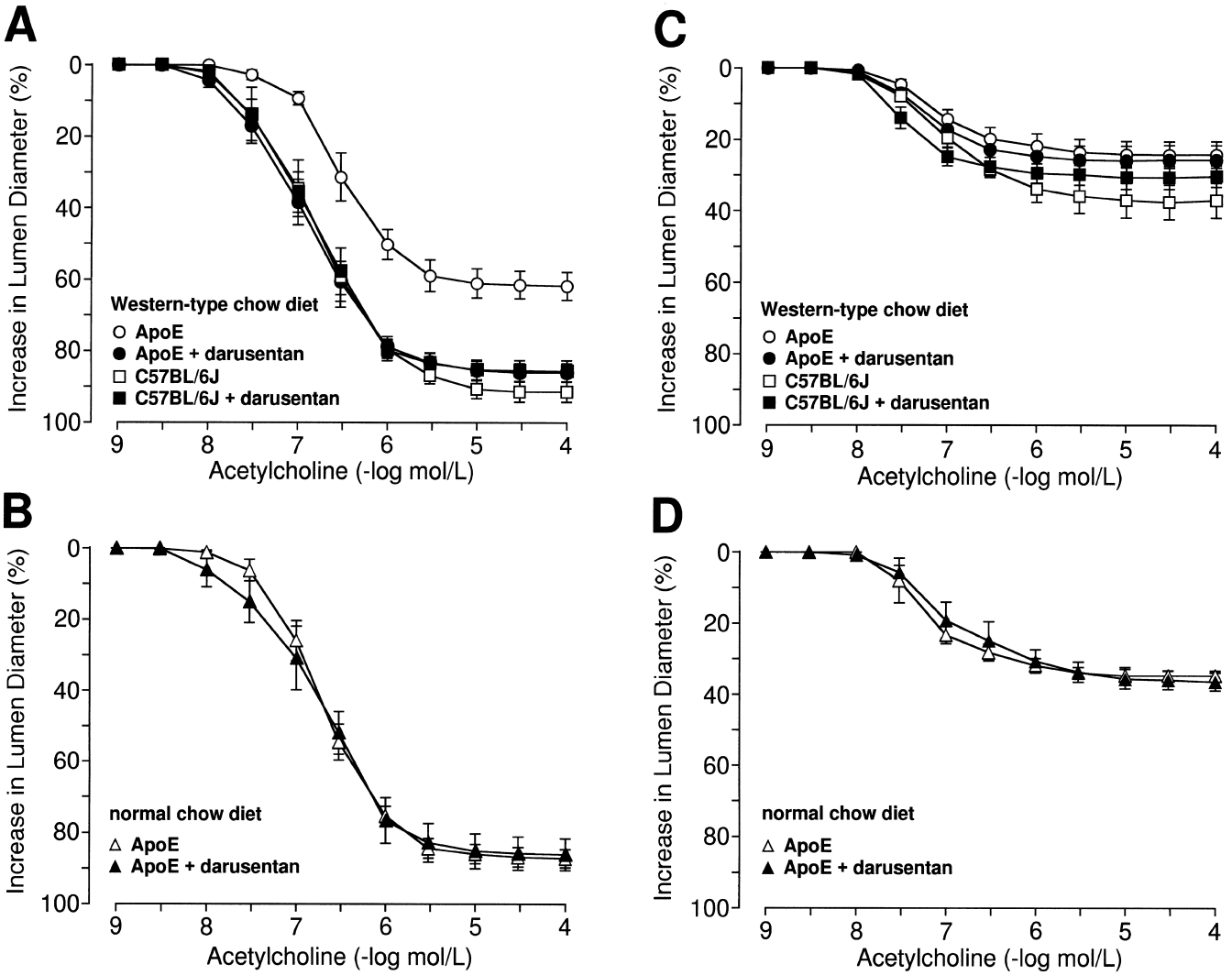

Fig. 2. Endothelium-dependent relaxations to acetylcholine in small mesenteric arteries of apoE-deficient and control mice after 30 weeks treatment with Western-type diet (A and C) and normal diet (B and D) alone or in combination with the ET_A receptor antagonist darusentan. Please note that relaxations to acetylcholine were reduced to the same extent in all groups in the presence of L-NAME (C and D). Results are shown as mean±S.E.M. (n=5–13 per group) and relaxations are expressed as percent of the increase in intraluminal diameter of the precontraction to norepinephrine.

response in apoE-deficient mice (Fig. 5B; n=5–7 per group).

4. Discussion

This present study demonstrates that in small arteries of apoE-deficient mice fed Western-type diet tissue ET-1 content was elevated and was associated with increase in wall thickness and impairment of endothelium-dependent relaxation to acetylcholine. Chronic blockade of ET_A receptors with darusentan normalized NO-mediated portion of the endothelium-dependent relaxation to acetylcholine and reduced increased ET-1 production and vascular reactivity to ET-1 without affecting systolic blood pressure or cholesterol levels.

Mice homozygous for the inactivated apolipoprotein E (apoE) gene provide a new model of human atherosclerosis. These mice develop spontaneous hypercholesterolemia and aortic atherosclerosis [17–19]. Heart rate and blood pressure were comparable between apoE-deficient and control mice on Western-type diet, but a significant decrease in body weight was found, while heart weight significantly increased. This is in line with the recent report by Hartley et al. [25]. Interestingly, our study showed that heart weight-to-body weight ratio was increased in apoE-deficient mice on normal diet and this increase was unaffected by Western-type diet. In addition, treatment with darusentan had no effect on these parameters in control and apoE-deficient mice irrespective of diet suggesting that ET-1 does not play a major role to these phenotypic alterations in apoE-deficient mice.

An impaired endothelium-dependent relaxation in response to acetylcholine has been observed in the aorta

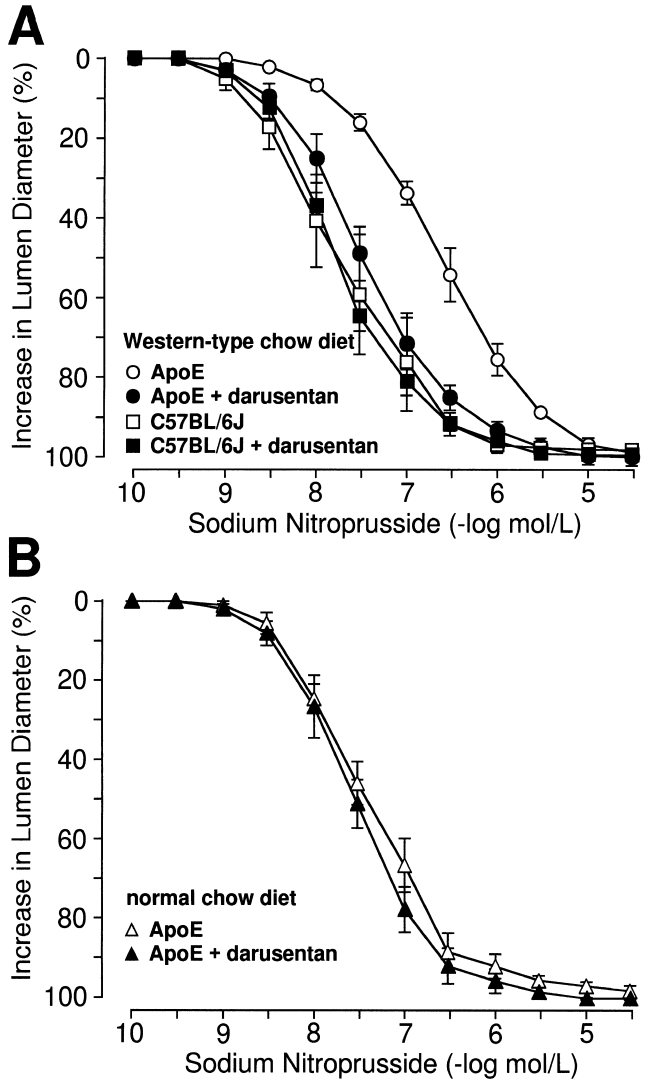

Fig. 3. Endothelium-independent relaxations to the nitric oxide donor sodium nitroprusside in small mesenteric arteries of apoE-deficient and control mice after 30 weeks treatment with Western-type diet (A) and normal diet (B) alone or in combination with the ET_A receptor antagonist darusentan. Results are shown as mean±S.E.M. (n=5–9 per group) and relaxations are expressed as percent of the increase in intraluminal diameter of the precontraction to norepinephrine.

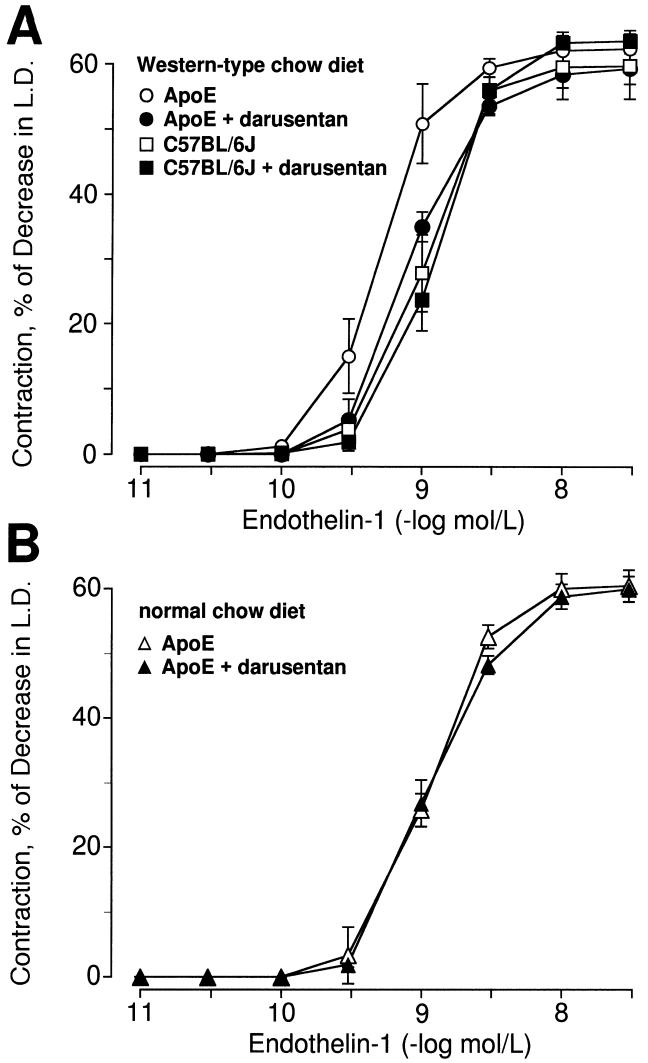

Fig. 4. Concentration-dependent contractions to ET-1 in small mesenteric arteries of apoE-deficient and control mice after 30 weeks treatment with Western-type diet (A) and normal diet (B) alone or in combination with the ET_A receptor antagonist darusentan. Results are shown as mean±S.E.M. (n=4–7 per group) and contractions are expressed as percent decrease of basal intraluminal diameter.

[26–29] and in coronary arteries of genetically altered hyperlipidemic mice [30]. Interestingly, although atherosclerosis primarily affects conduit arteries, endothelium-dependent relaxations are impaired not only in large arteries but also in small arteries of atherosclerotic animals and humans. In cremasteric vessels of cholesterol-fed rabbit cholinergic arteriolar vasodilation is defective [31]. In coronary arteries, hypercholesterolemia is associated with impaired endothelium-dependent relaxations to the agonists serotonin, bradykinin and histamine [13–16] indicating that the pathophysiological consequences of atherosclerosis may extend into small arteries also in humans. In our study, endothelium-dependent relaxations to acetylcholine were reduced in small arteries of apoE-deficient mice on Western-type diet. In contrast to the aorta [26,29], relaxation to acetylcholine in mesenteric arteries was inhibited only in part by L-NAME. The remaining portion of the response was not further attenuated by indomethacin, suggesting that an endothelium-dependent factor distinct from NO mediate part of relaxations to acetylcholine in mouse mesenteric arteries. Atherosclerosis had no effect on the L-NAME/indomethacin-insensitive portion of the response, contrasting a previous study that reported an augmentation of L-NAME/indomethacin-resistant relaxations to acetylcholine in renal arteries of hypercholesterolemic rabbits [32]. The discrepancy between these findings may be related to species differences and/or anatomic heterogeneity of vascular function. Furthermore, endothelium-dependent relaxations to acetylcholine were

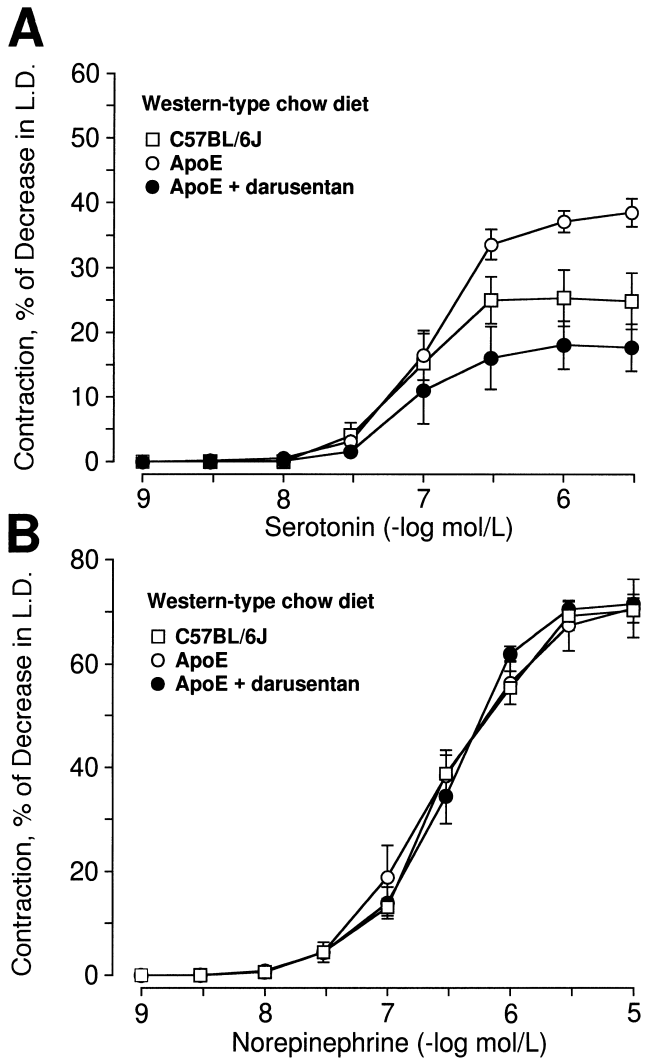

Fig. 5. Concentration-dependent contractions to serotonin (A) and norepinephrine (B) in small mesenteric arteries of apoE-deficient mice as compared with control C57BL/6J mice on Western-type diet. Results are shown as mean±S.E.M. (n=5–9 per group) and contractions are expressed as percent decrease of basal intraluminal diameter.

unaffected in the presence of indomethacin suggesting that endothelium-derived prostanoids do not play a major role in endothelial dysfunction. This is further confirmed by a recent study showing that no change in cyclic adenosine monophosphate occurred in the aorta of apoE-deficient mice [29].

Interestingly, chronic treatment with ET_A receptor antagonist darusentan normalized impaired endothelium-dependent and -independent relaxations in small mesenteric arteries. However, only the L-NAME-sensitive portion of the response to acetylcholine was increased indicating that the improvement of vascular reactivity was due to increased bioactivity of NO. In line with these observations, we recently reported that plasma nitrate levels, an indicator of NO activity in vivo, are increased in darusentan-treated apoE-deficient mice [26]. Possible mechanisms may include up-regulation of endothelial NO synthase protein, as has been reported after chronic ET_A blockade in hypercholesterolemic pigs [33,34]. In addition, the improvement in endothelium-dependent relaxations also appears to be related to an improved sensitivity of vascular smooth muscle cells to NO, as illustrated by the results obtained with the NO donor SNP.

ET-1 synthesis is stimulated by vasoactive factors and cytokines implicated in hypercholesterolemia [3]. ET-1 also stimulates cell proliferation and acts as a co-mitogen for vascular smooth muscle cells with other growth factors [6,35], suggesting that ET-1 contribute to structural changes through activation of multiple pathways. Importantly, ET-1 induces vascular hypertrophy is independent of systolic blood pressure [6,21] and in addition, ET_A receptors promote the early inflammatory phase of atherosclerosis [36]. In our study, vascular ET-1 peptide content and contractions to ET-1 were increased in small mesenteric arteries of apoE-deficient mice as compared with control animals on Western-type diet. Increased contractions to ET-1 may be explained by the activation of signal transduction pathways of ET-receptors. In addition, we previously have shown that ET_A receptor density in apoE-deficient mice aortas was increased [26]. The impairment of relaxation to acetylcholine was inversely related to vascular tissue ET-1 peptide content. Indeed, in apoE-deficient mice on normal diet neither endothelial dysfunction nor elevated tissue ET-1 content was found, despite a slight increase in plasma cholesterol levels. These observations strongly support a role for local ET-1 as an early participant in hypercholesterolemia and marker for endothelial dysfunction in animals and humans [7,8,33].

Interestingly, the ET_A receptor antagonist darusentan markedly reduced tissue ET-1 levels and prevent structural changes in small mesenteric arteries, indicating that endogenous ET-1 contributes to endothelial dysfunction. As no effects on blood pressure or plasma cholesterol were observed, the local effects of ET-1 appear to be independent of these risk factors. This is in line with the most recent study showing that blockade of ET receptors facilitates the maintenance of vasodilation despite high insulin concentrations [37]. In addition, darusentan treatment increased plasma HDL levels in control mice but not in apoE-deficient mice on Western-type diet again suggesting that improvement of endothelial dysfunction in apoE-deficient mice by darusentan treatment was due to the decreased local ET-1 production rather than increased production of plasma HDL. The reason for HDL increase by darusentan in control mice is unknown and remains to be determined.

Enhanced vasoconstriction to serotonin, which is an important factor aggravating ischemia in patients with coronary artery disease, is present in animals and patients with hypercholesterolemia and atherosclerosis [38–41]. In coronary arteries of apoE-deficient mice, fed a high cholesterol diet, was associated with a reduction of endo-

thelium-dependent relaxation to serotonin [30]. We here demonstrate that in addition to impaired endothelium-dependent relaxation in small mesenteric arteries, the contractions to serotonin were enhanced while contractions to norepinephrine remained unaffected in apoE-deficient mice compared to control mice on Western-type diet. This is confirmed by a recent study showing that deletion of eNOS gene in mice is associated with enhanced contraction to serotonin [42]. On the other hand, in isolated human arteries sub-threshold concentrations of ET-1 potentiate contractions to serotonin [43]. Thus, increased tissue ET-1 and endothelial dysfunction in mesenteric arteries may selectively augment serotonin-mediated vasoconstriction. Most interestingly, enhanced vasoconstriction was prevented by chronic ET_A receptor blockade, suggesting a role for ET_A receptors and/or local ET-1 tissue levels on serotonin-mediated vasoconstriction.

Improvement of vascular function and the reduction of wall thickness after chronic ET_A receptor blockade was associated with decreased vascular ET-1 tissue levels comparable to levels found in C57BL/6J control mice. Lerman et al. reported a strong correlation between ET-1 immunoreactivity in the coronary and systemic circulation and the severity of endothelial dysfunction in hypercholesterolemic animals and also in humans [7,8]. The mechanisms by which darusentan normalized elevated ET-1 tissue levels may include ET_A-mediated autocrine regulation of ET-1 mRNA [44] or protein synthesis in vivo [4,45] and/or interactions with tissue-bound ET-1 [6]. The beneficial effect of chronic ET_A receptor blockade on endothelial function in the vasculature may have important implications for local tissue perfusion and organ function, particularly if atherosclerosis is already present [14,15,46]. The findings suggest that darusentan normalized enhanced vasoreactivity to serotonin and ET-1 and improved endothelium-dependent vasomotion could be clinically relevant by adding novel anti-ischaemic properties to the growth-inhibiting effects of chronic endothelin blockade on structural changes.

Acknowledgements

Supported by the Swiss National Science Foundation (Grants No. 32-51069.97/1 to TFL, No. 32-49648.96 to SS and SCORE 32-58421.99 to MB), the ADUMED Foundation (to LVU and MB), the Novartis Foundation (to LVU) and a grant-in-aid by Knoll AG, Ludwigshafen, Germany. The authors are much indebted to Jane Boden for technical assistance and to Christian Binggeli, M.D. for MATLAB® software programming.

References

[1] Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987;327:524–526.

[2] Yanagisawa M, Kurihara H, Kimura S et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 1988;332:411–415.

[3] Miyauchi T, Masaki T. Pathophysiology of endothelin in the cardiovascular system. Annu Rev Physiol 1999;61:391–415.

[4] Alberts GF, Peifley KA, Johns A, Kleha JF, Winkles JA. Constitutive endothelin-1 overexpression promotes smooth muscle cell proliferation via an external autocrine loop. J Biol Chem 1994;269:10112–10118.

[5] Komuro I, Kurihara H, Sugiyama T, Yoshizumi M, Takaku F, Yazaki Y. Endothelin stimulates c-fos and c-myc expression and proliferation of vascular smooth muscle cells. FEBS Lett 1988;238:249–252.

[6] Moreau P, d'Uscio LV, Shaw S, Takase H, Barton M, Lüscher TF. Angiotensin II increases tissue endothelin and induces vascular hypertrophy: reversal by ET_A-receptor antagonist. Circulation 1997;96:1593–1597.

[7] Lerman A, Webster MW, Chesebro JH et al. Circulating and tissue endothelin immunoreactivity in hypercholesterolemic pigs. Circulation 1993;88:2923–2928.

[8] Lerman A, Holmes Jr. DR, Bell MR, Garratt KN, Nishimura RA, Burnett Jr. JC. Endothelin in coronary endothelial dysfunction and early atherosclerosis in humans. Circulation 1995;92:2426–2431.

[9] Boulanger C, Lüscher TF. Release of endothelin from the porcine aorta: Inhibition by endothelium-derived nitric oxide. J Clin Invest 1990;85:587–590.

[10] Dohi Y, Lüscher TF. Aging differentially affects direct and indirect actions of endothelin-1 in perfused mesenteric arteries of the rat. Br J Pharmacol 1990;100:889–893.

[11] Lüscher TF, Yang Z, Tschudi M et al. Interaction between endothelin-1 and endothelium-derived relaxing factor in human arteries and veins. Circ Res 1990;66:1088–1094.

[12] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801–809.

[13] Chilian WM, Dellsperger KC, Layne SM et al. Effects of atherosclerosis on the coronary microcirculation. Am J Physiol 1990;258:H529–H539.

[14] Sellke FW, Armstrong ML, Harrison DG. Endothelium-dependent vascular relaxation is abnormal in the coronary microcirculation of atherosclerotic primates. Circulation 1990;81:1586–1593.

[15] Zeiher AM, Drexler H, Wollschlager H, Just H. Endothelial dysfunction of the coronary microvasculature is associated with coronary blood flow regulation in patients with early atherosclerosis. Circulation 1991;84:1984–1992.

[16] Kuo L, Davis MJ, Cannon MS, Chilian WM. Pathophysiological consequences of atherosclerosis extend into the coronary microcirculation. Restoration of endothelium-dependent responses by L-arginine. Circ Res 1992;70:465–476.

[17] Plump AS, Smith JD, Hayek T et al. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 1992;71:343–353.

[18] Zhang SH, Reddick RL, Piedrahita JA, Maeda N. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science 1992;258:468–471.

[19] Breslow JL. Mouse models of atherosclerosis. Science 1996;272:685–688.

[20] d'Uscio LV, Moreau P, Shaw S, Takase H, Barton M, Lüscher TF. Effects of chronic ET_A-receptor blockade in angiotensin II-induced hypertension. Hypertension 1997;29:435–441.

[21] Barton M, d'Uscio LV, Shaw S, Meyer P, Moreau P, Lüscher TF. ET_A-receptor blockade prevents increased tissue endothelin-1, vascular hypertrophy and endothelial dysfunction in salt-sensitive hypertension. Hypertension 1998;31:499–504.

[22] De Lean A, Munson PJ, Rodbard D. Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose–response curves. Am J Physiol 1978;235:E97–E102.

[23] Heagerty AM, Aalkjær C, Bund SJ, Korsgaard N, Mulvany MJ.

Small artery structure in hypertension: dual processes of remodeling and growth. Hypertension 1993;21:391–397.
[24] Moreau P, Takase H, d'Uscio LV, Lüscher TF. Effect of chronic nitric oxide deficiency on angiotensin II-induced hypertrophy of rat basilar artery. Stroke 1998;29:1031–1035.
[25] Hartley CJ, Reddy AK, Madala S et al. Hemodynamic changes in apolipoprotein E-knockout mice. Am J Physiol Heart Circ Physiol 1999;279:H2326–H2334.
[26] Barton M, Haudenschild CC, d'Uscio LV, Shaw S, Münter K, Lüscher TF. Endothelin ET_A receptor blockade restores NO-mediated endothelial dysfunction and inhibits atherosclerosis in apoE-deficient mice. Proc Natl Acad Sci USA 1998;95:14367–14372.
[27] Bonthu S, Heistad DD, Chappell DA, Lamping KG, Faraci FM. Atherosclerosis, vascular remodeling and impairment of endothelium-dependent relaxation in genetically altered hyperlipidemic mice. Arterioscler Thromb Vasc Biol 1997;17:2333–2340.
[28] Kauser K, da Cunha V, Fitch R, Mallari C, Rubanyi GM. Role of endogenous nitric oxide in progression of atherosclerosis in apolipoprotein E-deficient mice. Am J Physiol Heart Circ Physiol 2000;278:H1679–H1685.
[29] d'Uscio LV, Baker TA, Mantilla CB et al. Mechanism of endothelial dysfunction in apolipoprotein E–deficient mice. Arterioscler Thromb Vasc Biol 2001;21:1017–1022.
[30] Lamping KG, Nuno DW, Chappell DA, Faraci FM. Agonist-specific impairment of coronary vascular function in genetically altered, hyperlipidemic mice. Am J Physiol 1999;276:R1023–R1029.
[31] Yamamoto H, Bossaller C, Cartwright Jr. J, Henry PD. Videomicroscopic demonstration of defective cholinergic arteriolar vasodilation in atherosclerotic rabbit. J Clin Invest 1988;81:1752–1758.
[32] Brandes RP, Behra A, Lebherz C et al. *N(G)*-Nitro-L-arginine- and indomethacin-resistant endothelium-dependent relaxation in the rabbit renal artery: effect of hypercholesterolemia. Atherosclerosis 1997;135:49–55.
[33] Best PJM, Lerman LO, Romero JC, Richardson D, Holmes DR, Lerman A. Coronary endothelial function is preserved with chronic endothelin receptor antagonism in experimental hypercholesterolemia in vitro. Arterioscler Thromb Vasc Biol 1999;19:2769–2775.
[34] Verhaar MC, Strachan FE, Newby DE et al. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. Circulation 1998;97:752–756.
[35] Peifley KA, Winkles JA. Angiotensin II and endothelin-1 increase fibroblast growth factor-2 mRNA expression in vascular smooth muscle cells. Biochem Biophys Res Commun 1998;242:202–208.
[36] Kowala MC, Rose PM, Stein PD et al. Selective blockade of the endothelin subtype A receptor decreases early atherosclerosis in hamsters fed cholesterol. Am J Pathol 1995;146:819–826.
[37] Misurski DA, Wu SQ, McNeill JR, Wilson TW, Gopalakrishnan V. Insulin-induced biphasic responses in rat mesenteric vascular bed: role of endothelin. Hypertension 2001;37:1298–1302.
[38] Henry PD, Yokoyama M. Supersensitivity of atherosclerotic rabbit aorta to ergonovine. Mediation by a serotonergic mechanism. J Clin Invest 1980;66:306–313.
[39] Shimokawa H, Tomoike H, Nabeyama S et al. Coronary artery spasm induced in atherosclerotic miniature swine. Science 1983;221:560–562.
[40] Heistad DD, Armstrong ML, Marcus ML, Piegors DJ, Mark AL. Augmented responses to vasoconstrictor stimuli in hypercholesterolemic and atherosclerotic monkeys. Circ Res 1984;54:711–718.
[41] Ludmer PL, Selwyn AP, Shook TL et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. New Engl J Med 1986;315:1046–1051.
[42] Lamping KG, Faraci FM. Role of sex differences and effects of endothelial NO synthase deficiency in responses of carotid arteries to serotonin. Arterioscler Thromb Vasc Biol 2001;21:523–528.
[43] Yang Z, Richard V, Segesser L et al. Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries: A new mechanism of vasospasm? Circulation 1990;82:188–195.
[44] Sakai S, Miyauchi T, Yamaguchi I. Long-term endothelin receptor antagonist administration improves alterations in expression of various cardiac genes in failing myocardium of rats with heart failure. Circulation 2000;101:2849–2853.
[45] Fujisaki H, Ito H, Hirata Y et al. Natriuretic peptides inhibit angiotensin II-induced proliferation of rat cardiac fibroblasts by blocking endothelin-1 gene expression. J Clin Invest 1995;96:1059–1065.
[46] Fujise K, Stacy L, Beck P et al. Differential effects of endothelin receptor activation on cyclic flow variations in rat mesenteric arteries. Circulation 1997;96:3641–3646.