Exercise Training Enhances Flow-Mediated Dilation in Spontaneously Hypertensive Rats

Filiz Gündüz[1], Günnur Koçer[1], Seher Ülker[1],

Herbert J. Meiselman[2] Prof, Oğuz K. Başkurt[3],

Ümit K. Şentürk[1]

[1]Akdeniz University, Faculty of Medicine, Department of Physiology, Antalya, Turkey

[2]University of Southern California, Keck School of Medicine, Department of Physiology and Biophysics, Los Angeles, CA, USA

[3]Koc University School of Medicine, Rumelifeneri Yolu, Istanbul, Turkey

Short title*:* Training enhances FMD in SHR

Correspondence

Address: Filiz Gündüz
Akdeniz University, Medical Faculty, Department of Physiology,
Kampus, 07070, Antalya, TURKEY

Telephone: +90 242 2496959

Fax: +90 242 2274483

E-mail: fgunduz@akdeniz.edu.tr

Summary

This study investigated the effect of exercise training on that flow-mediated dilation (FMD) in gastrocnemius muscle arteries from spontaneously hypertensive rats (SHR). SHR and WKY rats were divided into sedentary and exercised groups. After swimming exercise for eight weeks, the isolated arteries were mounted on pressurized myograph and FMD responses examined. The role of nitric oxide (NO), prostaglandins (PGs) and endothelium derived hyperpolarizing factor (EDHF) on FMD were assessed by obtaining dilation responses in the presence and absence of pharmacological antagonists. N^{ω}-nitro-L-arginine methyl ester (L-NAME), indomethacin (INDO) and tetraethylamonium (TEA) were used to inhibit nitric oxide synthase, cyclooxygenase and EDHF-mediated responses, respectively. The FMD response was significantly blunted in arteries of SHR compared with WKY rats, and, improved by exercise training in SHR (SHR-ET) group. In SHR, L-NAME and TEA did not affect dilation responses to flow of arteries while INDO led to a significant enhancement in this response. Although dilation response was not altered by L-NAME in arteries obtained from trained SHR, TEA caused a significant attenuation and INDO led to significant increases. These results demonstrate that exercise training improves FMD in SHR, and, this enhancement induced by exercise training occurs through EDHF-mediated mechanism(s).

Key words: EDHF; nitric oxide; prostaglandins

Introduction

The endothelium plays an important role in the modulation of vascular tonus through the production of numerous vasoactive substances. Several neurohumoral agents and mechanical forces such as shear stress that occur during blood flowing contribute to endothelium-dependent dilation (Mulvany and Aalkjaer 1990). Flow-induced shear stress regulates endothelial responses by modulating the release of endogenous factors such as nitric oxide (NO), prostaglandins (PGs) and results in flow-mediated dilation (FMD). On the other hand, endothelium derived hyperpolarizing factor (EDHF) is another important mediator that involve in FMD, thereby in endothelium-dependent dilation (Koller *et al.* 1994, Takamura *et al.*1999). It's generally accepted that EDHF-mediated vasodilatory responses is associated with endothelial and smooth muscle cells hyperpolarization by opening potassium (K^+) channels, without release any vasoactive factor (Nagao and Vanhoutte 1993).

Endothelial dysfunction that results from impaired endothelium-dependent dilation contributes to increased peripheral vascular resistance in hypertension (Folkow 1982, Priviero *et al*. 2009). Decreased relaxation response to both of neurohumoral factors and flow has been shown in many animal models of hypertension and humans (Higashi and Yoshizumi 2004, Koller and Huang 1994, Kuru *et al.* 2009, Priviero *et al*. 2009). Although the underlying mechanism or mechanisms are controversial, several investigations have also demonstrated that vascular dilation response to increases in flow is attenuated in small arteries responsible for peripheral vascular resistance in spontaneously hypertensive rats (SHR) that is an animal model simulating human essential hypertension (Koller and Huang 1994, Koller and Huang 1999, Matrougui *et al.* 1997, Qiu *et al.* 1998).

Exercise training improves endothelial function in hypertension (Chen *et al*. 1996, Higashi *et al*. 1999, Kuru *et al.* 2009). The most frequently proposed mechanism for this effect of exercise on endothelial function is the increased vascular blood flow and shear stress

that stimulates endothelial nitric oxide synthase (eNOS)-dependent NO synthesis (Higashi and Yoshizumi 2004, Husain 2002). PGs and EDHF are other endothelium-dependent vasodilatory factors that may participate in exercise training-induced vasodilation (Higashi and Yoshizumi 2004). On the other hand, the effect of regularly physical exercise on endothelial function in SHR has been investigated, in partly. Several investigators have shown improved acetylcholine (ACh)-induced dilation responses in large and small artery segments obtained from exercised-trained SHR (Chen *et al*. 1996, Chen *et al*. 1999, Graham and Rush 2004, Yen *et al*. 1995). However, the possible influence of exercise on flow-mediated dilation in SHR has not yet been clarified. Because of ACh and flow trigger endothelium-dependent dilation by different mechanisms, evaluation of FMD is important for the assessment of endothelial function.

The aim of the present study was to investigate how exercise training affects the FMD in small arteries in SHR. We hypothesized that attenuated FMD responses could be improved by exercise training in SHR. To test our hypothesis, we investigated the changes in dilation responses as a function of perfusate flow in isolated gastrocnemius muscle arteries of trained or untrained-normotensive and hypertensive rats. In addition to assessing the possible role of NO, PGs and EDHF-mediated responses in the modulation of FMD by exercise training; we also evaluated FMD response in the presence of pharmacological agents that block the synthesis and/or activities of those mediators.

Methods

Male spontaneously hypertensive rats (SHR) at 11-12 weeks of age and age-matched normotensive Wistar Kyoto (WKY) rats (Harlan Laboratories, USA) were used in the present study. The animals were housed at 23±2°C on a 12:12 h light-dark cycle and had free access to standard rat chow and drinking water. Rats were assigned randomly to four different groups: WKY sedentary (WKY, n=9), WKY-exercise training (WKY-ET, n=9), SHR-

sedentary (SHR, n=8), and SHR- exercise training (SHR-ET, n=9). The animals in the exercise training groups were subjected to swimming exercise (60 min/day, five days/week for eight weeks) in a glass tank of 100 x 50 cm with a depth of 50 cm filled with tap water (32-34°C). The duration of the first swimming experience was limited to 10 min and increased by 10 min daily until 60 min was reached. The experimental protocol was approved by the Animal Care and Usage Committee of Akdeniz University and was in accordance with the Declaration of Helsinki and International Association for the Study of Pain (IASP) guidelines.

The systolic blood pressure of all rats was measured using a non-invasive tail-cuff method at the beginning of the study (basal) and every two weeks during the eight week period. Data were obtained with a MAY-BPHR 9610-PC unit and MP 150 data acquisition system (BIOPAC Systems; Santa Barbara, CA-USA). The final measurements were performed one day after the last swimming session in exercising animals.

Isolation of feed arteries

Rats were anesthetized with an intraperitoneal injection of thiopental sodium (80 mg/kg body weight) one day after the last exercise period in the training groups. The gastrocnemius-soleus muscle group was removed and transferred to a dissecting dish filled with ice-cold physiological saline solution (PSS) containing (in mM) 145.0 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.17 $MgSO_4$, 1.2 NaH_2PO_4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 25.0 MOPS, pH=7.4.

The gastrocnemius feed arteries (~200 μm in diameter) were carefully dissected free under a dissecting microscope (SZ61, OLYMPUS; Tokyo, Japan). The isolated arterial segments were transferred to a vessel chamber (CH/1, Living Systems, Inc., Burlington, VT, USA) containing two horizontal glass micropipettes filled with PSS-albumin (1 g/100 mL). After the vessel was mounted on the proximal pipette and secured with 11-0 surgical nylon

suture the perfusion pressure was raised to 20 mm Hg to clear clotted blood from the lumen. Then the other end of the vessel was mounted on the distal pipette. A pressure servo-controlled roller pump perfusion system (Living Systems, Inc., Burlington, VT) was connected to the proximal pipette, and a similar but manually-controlled roller pump attached to the distal end. The pressure in each pipette was monitored by pressure transducers, and thus the intraluminal pressure in the vessel could be controlled by the pressure servo-controlled perfusion system.

The mounted vessel segments were visualized by an inverted microscope (Eclipse TS100, Nikon) equipped with a charge-coupled device camera (XC73CE, Sony). The camera was connected to a video dimension-analysis system (model V94, Living Systems) which allowed continuous measurement of vessel diameter. Mean intraluminal pressure, pressure gradient, fluid flow rate through the arterial segment, and vessel diameter were continuously recorded via a data acquisition system (MP 100A-CE; BIOPAC Systems) connected to a personal computer.

The arteries were perfused with MOPS-PSS supplemented with albumin (1 g/100 mL) and the axial length of the arterial segment was adjusted by positioning the cannula until the vascular walls were parallel without obvious stretch. Vessels that were free from leaks were pressurized to 60 mm Hg with the servo-controlled pump, gradually warmed to 37 ºC, and allowed to develop spontaneous tone during equilibration period. The preparations were left to equilibrate for one h while the bathing solution was changed every 15 min.

Evaluation of flow mediated dilation responses

Flow mediated dilation (FMD) responses were assessed using various flow rates between 7-45 μL/min while keeping intraluminal pressure constant at 60 mm Hg by the pressure servo-controlled system. Each flow rate was maintained for five min to obtain a steady-state vessel diameter. After obtaining control responses the arterial segment was washed and the role of NO in the mediation of FMD responses was assessed. The relative

contribution of NO was evaluated by examining FMD responses in the presence of N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10^{-4} M), an inhibitor of NOS. Vessels were incubated with L-NAME for 20 min and than FMD responses were reassessed. The role of prostaglandins was assessed after 20 min incubation period with indomethacin (INDO, 10^{-5} M), an inhibitor of cyclooxygenase (COX). Finally, after washing the vessel, a K^+ channel blocker, tetraethylamonium (TEA, 10^{-3} M) was added in the bath solution, and, after 20 min incubation period dilation responses were reevaluated to determine the role of non-NOS and non-COX pathways in the FMD.

At the end of experiment, the MOPS-PSS bath solution was replaced with Ca^{++} -free PSS and the vessels were incubated at least for 30 min to determine their maximal passive diameter.

Statistics

All values are given as means ± standard error of the mean (SEM). Changes in diameter in response to increases in the perfusate flow were normalized to the corresponding passive diameter and expressed as percent maximal response by using following calculation: $(D_d - D_b)/ (D_p - D_b)$ x 100 where D_d is the measured diameter for a given flow; D_b is the baseline diameter before an intervention was started, and D_p is the maximal passive diameter. Initial tone is expressed as a percentage of maximal passive diameters. Between-group differences in blood pressure, maximal passive diameter and the initial tone of vessels from WKY and SHR rats were assessed using one-way ANOVA. Two-way ANOVA with repeated measures was used for comparison of the flow-dilation response curves and blood pressure levels; the Bonferroni test was used as a post-hoc test. P values <0.05 were considered significant.

Results

Systolic blood pressure (SBP) levels were elevated in SHR compared to WKY rats and exercise training induced a significant decrease in blood pressure in SHR compared to the untrained-SHR group (Table). The difference became significant at the 4th week of exercise and continued untill the end of experiment. There was no significant change in SBP in exercise-trained normotensive rats (WKY-ET). Maximal passive diameters and initial tonus of gastrocnemius arteries were similar in all groups (data not shown).

Vasodilation responses to flow and effect of exercise training. Vasodilation in response to intraluminal flow was decreased in gastrocnemius arteries from SHR compared to those from WKY rats. Exercise resulted in a significant improvement in dilation response to flow in SHR but not in WKY rats (Fig 1).

Effect of NOS inhibition. NOS inhibition with L-NAME diminished FMD in gastrocnemius arteries for both WKY and WKY-ET rats (Fig 2A and B). However, dilation responses to flow were not altered by NOS inhibition with L-NAME in the SHR and SHR-ET groups (Fig 2C and D).

Effect of COX inhibition. INDO significantly reduced FMD in gastrocnemius arteries of both untrained and trained normotensive rats (Fig 3A and B). However, in hypertensive rats, COX inhibition by INDO caused significant increases in FMD, mainly for the non-exercised SHR rats (Fig 3C and D).

Effect of potassium channel inhibition. Dilation responses to increase in perfusate flow in arteries from WKY and WKY-ET rats were significantly decreased after K^+ channel inhibition with TEA (Fig 4A and B). TEA did not alter FMD in arteries of the SHR group (Fig 4C) whereas it resulted in significantly decreased dilation responses in arteries obtained from SHR-ET (Fig 4 D).

Discussion

The aims of present study were: 1) to determine whether exercise training restores the attenuated FMD response in SHR; 2) to investigate possible mechanism or mechanisms that mediate an effect of exercise training. Our results clearly demonstrate that exercise training countered the reduction of FMD in gastrocnemius muscle arteries from SHR, and that this improvement appears to be linked to EDHF related mechanism(s).

It is known that altered behavior of vascular endothelial cells, as well as morphological changes of vascular wall, is involved in the development of hypertension (Folkow 1982, Mulvany 1993). Endothelial dysfunction has been well defined in SHR which is an animal model simulating human essential hypertension (Félétou *et al*. 2009). Reduced NO bioavailability, altered PGs production and/or efficacies are most proposed mechanisms that contribute to endothelial dysfunction and endothelium dependent contraction that is elicited by ACh in SHR aortas (Félétou *et al*. 2009). On the other hand the markedly decrease of EDHF-mediated responses has been shown by several studies in resistance arteries obtained from SHR (Mantelli *et al.* 1995, Mori *et al.* 2006). Thereby, the endothelial dysfunction observed in small arteries in SHR seems to be related with diminished EDHF mediated mechanism(s).

It has been suggested that the beneficial effect of exercise on endothelial function involves the blood pressure lowering effect of exercise (Higashi *et al*. 1999, Higashi and Yoshizumi 2004, Husain 2002, Kuru *et al.* 2009). The improvement of vascular dilation response to ACh in SHR also has been presented by previous studies (Chen *et al*. 1996, Yen *et al*. 1995). In our present investigation, we observed that swimming training caused a decrease of blood pressure in hypertensive rats beginning from the 4^{th} week of exercise through the end of the experiment (Table). Lowering blood pressure by physical exercise has been demonstrated in hypertensive rats by several investigators (Bertagnolli *et al.* 2008,

Horta *et al.* 2005, Kuru *et al.* 2002, Yen *et al*. 1995), although some studies did not confirm these findings (Graham and Rush 2004). These discrepancies may be explained by the different exercise (intensity, duration or kind) or age of animals used in those studies.

Impaired dilation response to flow has also been previously demonstrated in SHR small arteries (Koller and Huang 1994, Koller and Huang 1999, Matrougui *et al.* 1997, Qiu *et al.* 1998), and our results are consistent with these reports (Fig 1). On the other hand, it is not clear whether regularly physical activity affects the responses of small arteries to flow in SHR. To our knowledge, this is the first study investigating the possible exercise training-induced alterations in response to flow of resistance arteries from trained SHR. The primary finding of this study is the exercise training improves the FMD responses in gastrocnemius arteries from SHR (Fig 1). Additionally, the possible role of NOS, COX and/or EDHF related mechanisms in exercise training-induced improvement in FMD responses was also evaluated in the present study.

The role of NO production in the response to flow is well known (Koller *et al.* 1994). Flow-induced NO production is the most frequently proposed mechanism for the beneficial effect of exercise training on endothelial function (Higashi *et al*. 1999, Higashi and Yoshizumi 2004). In agreement with these observations, we also determined a significant decrease in FMD response after L-NAME treatment in both of WKY and WKY-ET rats (Fig 2A and B). However, the already-reduced dilation response to flow of SHR arteries was not further decreased after L-NAME incubation period (Fig 2C). The dilation response to flow was elevated by exercise training in SHR-ET, but this response also was not affected by NOS inhibition (Fig 2D). These results suggest that NO does not play an important role in FMD in genetically hypertensive rats.

The NO pathway is altered in hypertension. Prior studies have shown that the attenuated dilation response to flow in small arteries from SHR is insensitive to NO synthesis

blockade (Koller and Huang 1994, Koller and Huang 1999, Matrougui *et al.* 1997, Qiu *et al.* 1998). Although the mechanism underlying the attenuation is not yet clear, it has been proposed that impairment in the signal transduction that links flow or shear stress to NO release might be altered in SHR (Koller and Huang 1999). However, Qiu et al. (1998) demonstrated that although flow-induced NOS activity and cGMP release were significantly greater in mesenteric resistance arteries from SHR versus those of WKY, the dilation response to flow was markedly decreased in the SHR arteries. This decreased dilation response from these animals despite increased flow-induced cGMP production supports the idea that cGMP might be less efficient in SHR. On the other hand decreased NO bioavailability might be involved in endothelial dysfunction in hypertension (Priviero *et al.* 2009). Some studies suggest that increased superoxide anion and NO production in hypertension which may result in peroxynitrite formation and decreased NO bioavailability (Grunfeld 1995, Pechánová and Simko 2007).

Presumably, the participation of PGs in the dilation response to flow is variable depending on the anatomic localization of the vascular bed and/or the species (Friebel *et al.* 1995, Matrougui *et al.* 1997), similar to NO. Involvement of PGs in the dilation response was investigated using INDO, and our results demonstrated that PGs-induced dilation is a part of the FMD response from trained and untrained WKY rats (Fig 3A and B). This finding is in agreement with results obtained from rat skeletal muscle arterioles (Friebel *et al.* 1995, Koller *et al.* 1993). On the other hand, whether the flow-induced dilation is sensitive to PGs synthesis blockade in small arteries in SHR is still controversial. Koller and Huang (1994) showed that dilation response to flow decreased during PG synthesis blockade in gracilis muscle arterioles, whereas in another study, COX blockade did not affect this response in mesenteric resistance arteries, in SHR (Matrougui *et al.* 1997).

Interestingly, our results demonstrated that the dilation response to flow in muscle resistance arteries in SHR was augmented by PGs synthesis blockade (Fig 3C). This finding correlates with an impaired ACh-induced response being restored in SHR aorta and small mesenteric arteries by COX inhibition (Graham and Rush 2009, Lüscher *et al.* 1990). Although we could not evaluate vascular COX expression in this present study because the inadequate specimen, several previous studies have shown increased vascular production of constrictor PGs and increased vascular COX-1 expression in SHR (Ge *et al.* 1995, Huang *et al.* 2000), and, that these changes progress with age (Graham and Rush 2009, Tang and Vanhoutte 2008). Moreover, endothelial impairment, as well as increases in blood pressure, becomes more evident with age in genetically hypertensive rats (Koller and Huang 1999). The above mentioned studies (Koller and Huang 1994, Matrougui *et al.* 1997) were performed in 12 week-old SHR whereas our animals were about 20 weeks old at the time of the experiment because of the necessity of waiting for the eight week training period to conclude. When all these findings are taken together, it could be speculated that a possible age-related alteration(s) in PGs production might contribute to the endothelial dysfunction that is evident with aging in SHR. Although such as increase in FMD response at the presence of INDO was at a lesser extent in trained-SHR group, it was still significantly greater than those of SHR (Fig 3D). Thus our result suggests that PGs might not be involved in exercise-induced improvement of FMD in SHR.

EDHF related mechanism(s) is one of the important mediators that involved in endothelium-dependent vasodilation, and hyperpolarizes vascular smooth muscle cells by opening potassium channels (Nagao and Vanhoutte 1993, Shimokawa *et al.* 1996). It has been shown that EDHF-mediated vascular relaxation represents part of the flow or shear stress-induced dilation, and that it has physiological importance in both small and large arteries (Shimokawa *et al.* 1996). Although the role of NO and PGs in flow-induced dilation

in small arteries from SHR has been widely investigated (Friebel *et al.* 1995, Holtz *et al.* 1984, Koller *et al.* 1993) the role of EDHF remains to be elucidated.

In this present study, we used TEA, a nonselective potassium channel inhibitor, to investigate whether EDHF pathway contributes to the FMD response in our experimental groups. Our results demonstrated that TEA caused a significant reduction in response to flow in WKY and WKY-ET rats (Fig 4A and B) and are in agreement with results obtained in mesenteric small arteries of normotensive rats (Takamura *et al.* 1999). On the other hand, the FMD response was not influenced by potassium channels blockage in SHR whereas it was significantly reduced at 45 μL/min flow rate in SHR-ET (Fig 4C and D). This observation suggests that the EDHF pathway may also be altered in hypertensive rats, and, involved in the beneficial effect of exercise training on dilation response to flow in SHR. Although this is the first study that demonstrates the role of EDHF in exercise-induced flow-mediated vasodilation in SHR, it has been shown that EDHF related mechanism(s) contributes to ACh-induced dilation in mesenteric arteries from exercised-SHR species (Yen *et al.* 1995).

In summary, the results of this study indicate that dilation response to flow was improved by regularly exercise training in gastrocnemius arteries in SHR. This improvement effect of exercise training seems to be by means of EDHF-related mechanism(s)

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Table: Systolic blood pressure levels in normotensive and hypertensive rats during the eight week period.

	Weeks				
	0	**2**	**4**	**6**	**8**
	mm Hg				
WKY	129.8±1.1	130.6±1.4	130.3±1.6	131.1±1.1	133.8±1.1
WKY-ET	131.2±1.0	130.7± 1.1	131.6± 1.2	130.0± 1.5	133.1± 1.2
SHR	190.8± 1.3*	191.9±1.0*	195.8± 1.4*	199.1±1.2*	203.9± 1.2*
SHR-ET	192.0± 1.7*	188.8± 1.2*	188.4± 0.9*†	190.4± 1.0*†	183.7± 2.0*†

Values are given as the mean±SEM. *p<0.001 difference from WKY, †p<0.001 difference from SHR.

WKY, Wistar Kyoto; SHR, spontaneous hypertensive rat; WKY-ET, Wistar Kyoto exercise-trained; SHR-ET spontaneous hypertensive rat exercise-trained.

Figure 1: Flow-mediated dilation (FMD) response in gastrocnemius feed arteries from normotensive (WKY) and hypertensive (SHR) rats and the effect of exercise on FMD. Values are means ± SEM. *p<0.05, **p<0.01, ***p<0.001, difference from WKY; $^{\#}$p<0.05, difference from SHR, †p<0.05, difference from WKY-ET.

Figure 2: Effect of nitric oxide synthase (NOS) inhibition on flow-mediated dilation (FMD) response in gastrocnemius feed arteries. **A and B:** Effect of NOS inhibition on FMD response in WKY and WKY-ET. **C and D:** Effect of NOS inhibition on FMD response in SHR and SHR-ET. Values are means ± SEM. *p<0.05, **p<0.01, ***p<0.001, difference from WKY or WKY-ET.

Figure 3: Effect of cyclooxygenase (COX) inhibition on flow-mediated dilation (FMD) response in gastrocnemius feed arteries. **A and B:** Effect of COX inhibition on FMD response in WKY and WKY-ET. **C and D:** Effect of COX inhibition on FMD response in SHR and SHR-ET. Values are means ± SEM. *p<0.05, **p<0.01, ***p<0.001, difference from WKY or WKY-ET, SHR or SHR-ET.

Figure 4: Effect of endothelium-derived hyperpolarizing factor (EDHF) inhibition on flow-mediated dilation (FMD) response in gastrocnemius feed arteries. **A and B:** Effect of EDHF inhibition on FMD response in WKY and WKY-ET. **C and D:** Effect of EDHF inhibition on FMD response in SHR and SHR-ET. Values are means ± SEM. *p<0.05, **p<0.01, ***p<0.001, difference from WKY or WKY-ET or SHR-ET.

Figure 1

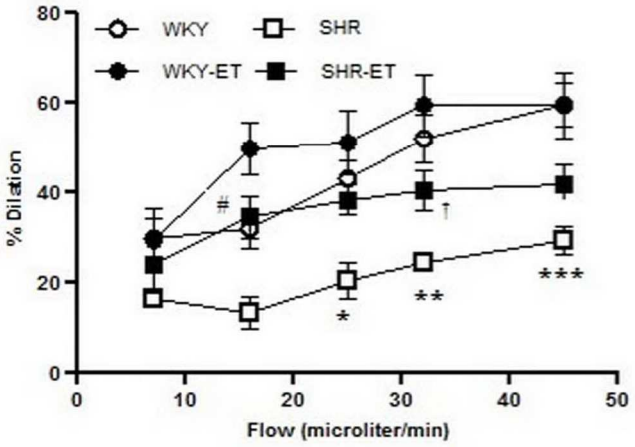

WKY
SHR
WKY-ET
SHR-ET
% Dilation
Flow (microliter/min)
0
20
40
60
80
0
10
20
30
40
50
#
*
**
***
†

Figure 2

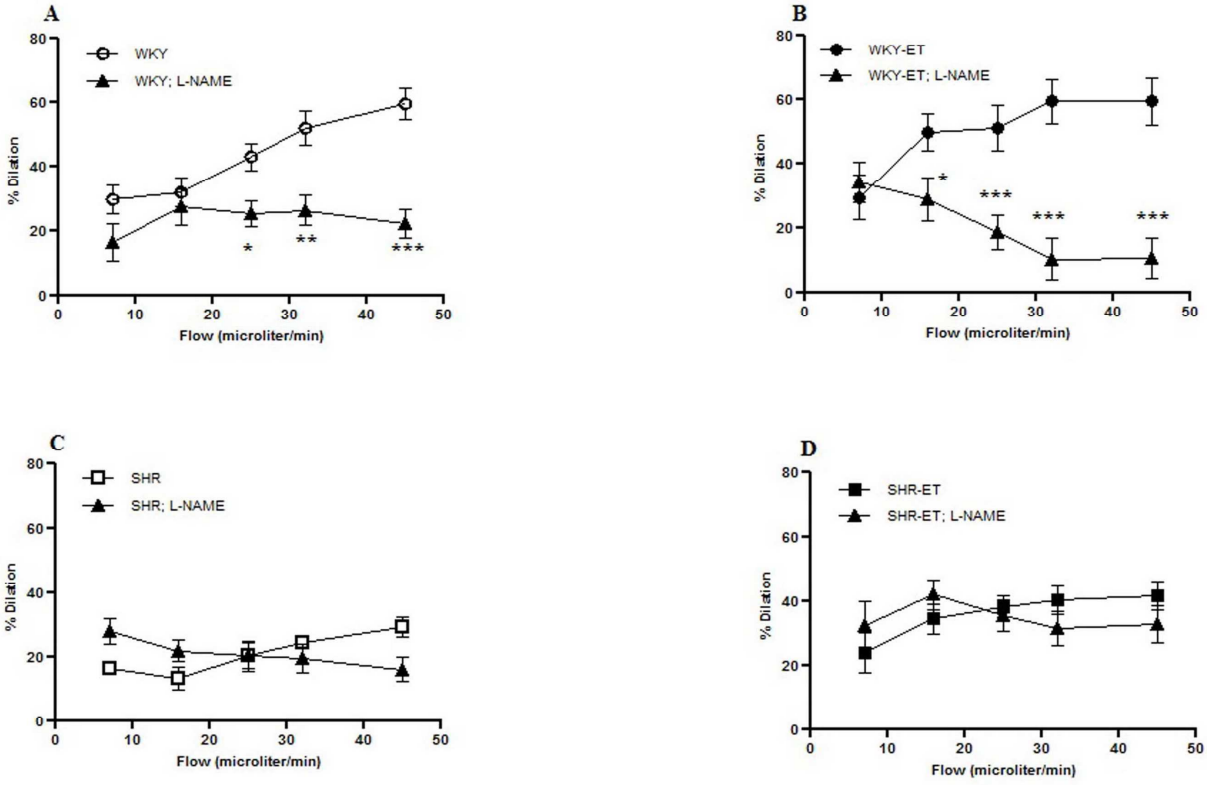

A
WKY
WKY; L-NAME
% Dilation
Flow (microliter/min)
B
WKY-ET
WKY-ET; L-NAME
% Dilation
Flow (microliter/min)
C
SHR
SHR; L-NAME
% Dilation
Flow (microliter/min)
D
SHR-ET
SHR-ET; L-NAME
% Dilation
Flow (microliter/min)

Figure 3

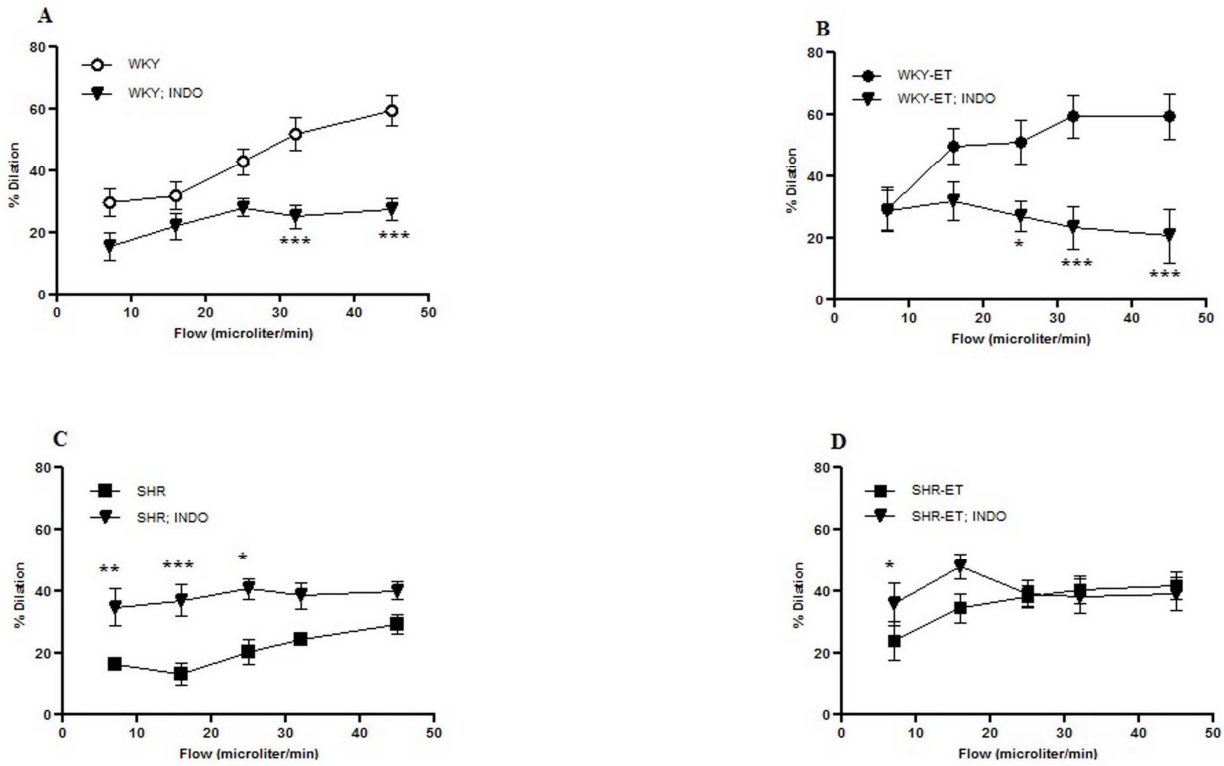
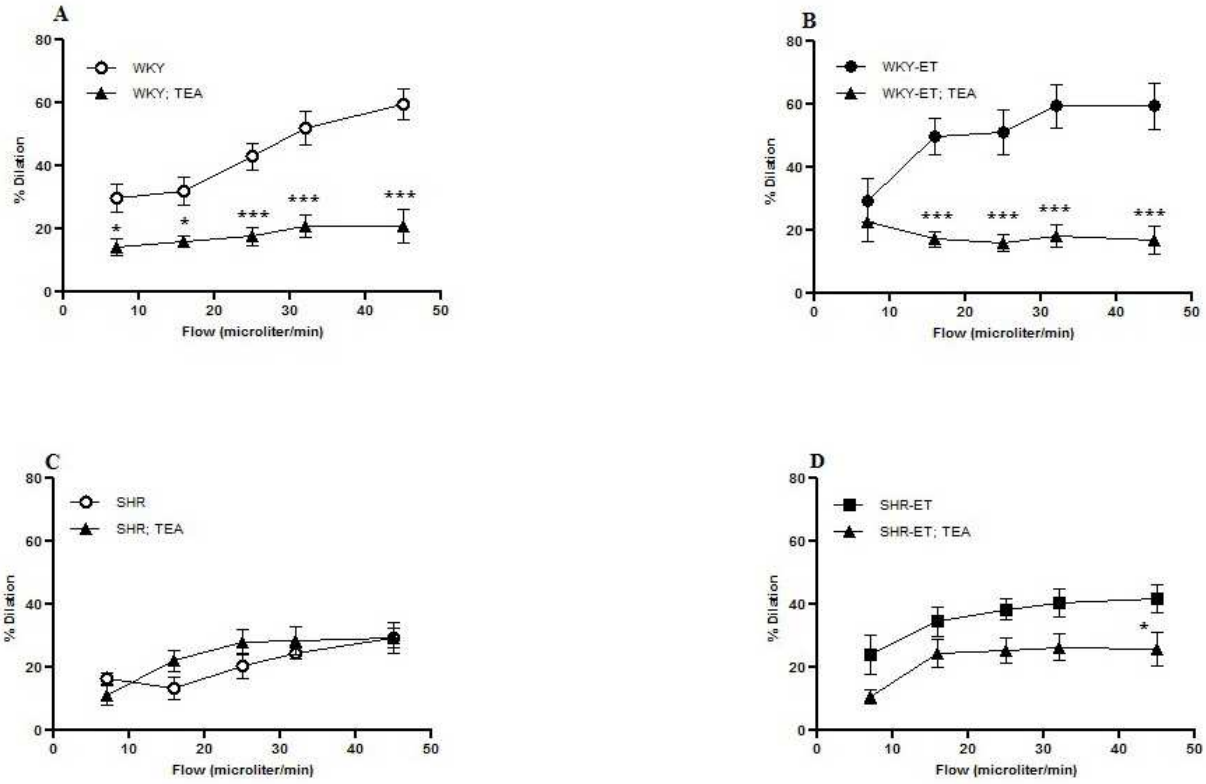

A
WKY
WKY; INDO
% Dilation
Flow (microliter/min)
***
***
B
WKY-ET
WKY-ET; INDO
% Dilation
Flow (microliter/min)
*
***
***
C
SHR
SHR; INDO
% Dilation
Flow (microliter/min)
**
***
*
D
SHR-ET
SHR-ET; INDO
% Dilation
Flow (microliter/min)
*

Figure 4


A
WKY
WKY; TEA
% Dilation
Flow (microliter/min)
B
WKY-ET
WKY-ET; TEA
% Dilation
Flow (microliter/min)
C
SHR
SHR; TEA
% Dilation
Flow (microliter/min)
D
SHR-ET
SHR-ET; TEA
% Dilation
Flow (microliter/min)