Chronic and Acute Effects of Different Antihypertensive Drugs on Femoral Artery Relaxation of L-NAME Hypertensive rats

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Running title: The effects of antihypertensive drugs on vasorelaxation

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Summary

We aimed to compare effects of chronic and acute administration of structurally different antihypertensives, diuretics - indapamide and hydrochlorothiazide, ACE inhibitor - captopril and indapamide+captopril combination on endothelium-dependent relaxation of femoral artery isolated from nitric oxide (NO)-deficient rats. In the chronic experiment, femoral artery was isolated from Wistar rats receiving L-NAME (40 mg/kg/day) solely or with indapamide (1 mg/kg/day), hydrochlorothiazide (10 mg/kg/day), captopril (10 mg/kg/day), and indapamide+captopril combination for seven weeks. In the acute experiment, incubation medium with femoral artery isolated from L-NAME-hypertensive rats was supplemented with investigated antihypertensives in the same concentration 10^{-4} mol/l. Interestingly, chronic L-NAME treatment did not lead to the reduction of vasorelaxation. Indapamide+captopril elevated relaxation above the control level and completely prevented blood pressure increase induced by L-NAME. Acute incubation only with captopril and indapamide+captopril improved relaxation of femoral artery isolated from L-NAME-hypertensive rats, while incubation with all antihypertensives investigated increased vasorelaxation of femoral artery isolated from control Wistar rats. In conclusion, NO might be involved in the indapamide- and hydrochlorothiazide-induced improvement of vasorelaxation while different vasorelaxing factors (prostacyclin, EDHF) in the captopril-induced improvement of vasorelaxation. During the chronic treatment additive and synergic effects of indapamide and captopril may contribute to the prevention of hypertension and increase of vasorelaxation.

Key words: L-NAME-induced hypertension • indapamide • hydrochlorothiazide • captopril • femoral artery • vasorelaxation

Introduction

Hypertension remains one of the leading causes of morbidity and mortality in the most of developed countries. Many hypertensive patients suffer from concomitant diseases or conditions associated with high blood pressure (Šimko 2002, 2007). Quite complex analysis is required to pick the initial and additional antihypertensive agents that are simultaneously beneficial in the management of these comorbidities. Much data on the safety and value of diuretics, beta blockers, calcium-channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers in reducing blood pressure and preventing clinical disease have been accumulated now (Cohn 2001).

While studying effects of different antihypertensives, it seems to be substantially important to analyse their interference with the L-arginine – nitric oxide pathway, renin-angiotensine-aldosteron system, sympathetic nervous system and oxidative status. Protection of physiological function of these systems may reduce cardiovascular remodeling and renal damage (Šimko *et al.* 2003, Zicha *et al.* 2006a, Pecháňová *et al.* 2006, Kojšová *et al.* 2006, Kristek *et al.* 2007).

ACE inhibitors and diuretics belong to the important tools in blood pressure reduction. ACE inhibitor, captopril inhibits angiotensin II formation resulting in blood pressure decrease, vasorelaxation (Johns *et al*. 1984, Török *et al*. 2002), cardioprotection (Šimko and Šimko 1990, Konstam *et al*. 2000, Bernátová *et al.* 2000) and renoprotection (Manley 2000, Pecháňová *et al.* 2006). In contrast to hydrochlorothiazide, a thiazide-like diuretic indapamide has been suggested to possess vasorelaxing and antioxidant properties besides its diuretic effect (Uehara *et al.* 1990,

Kojšová *et al.* 2006). Experimental studies point to the fact that reduction of blood pressure and prevention of left ventricular hypertrophy development, induced by indapamide treatment, is associated with increase of NO synthase activity (Hayakawa *et al*. 1997).

The aim of our study was to evaluate the acetylcholine-induced relaxation of femoral artery using NO-deficient model of hypertension, particularly the model of N^G-nitro-L-arginine methyl ester (L-NAME)-induced hypertension. The effects of treatment with indapamide or hydrochlorothiazide or captopril or indapamide+captopril combination on the prevention of L-NAME-induced hypertension and *arteria femoralis* relaxation were studied. Furthermore, the acute effects of the investigated antihypertensives on the femoral artery relaxation were analysed.

Material and Methods

Animals and treatment

All procedures and experimental protocols were approved by the Ethical Committee of the Institute of Normal and Pathological Physiology SAS, and conform to the European Convention on Animal Protection and Guidelines on Research Animal Use. The animals were housed in an air-conditioned room at a stable temperature (22-24 °C) and humidity (45-60%) on a 12:12 light/dark cycle and maintained on a standard pellet diet and tap water *ad libitum*. Daily water consumption was estimated one week before the experiment and controlled during the treatment.

In the chronic treatment, adult 12-week-old Wistar rats were divided into six groups: control (n=6), group treated with N^G-nitro-L-arginine methyl ester (L-NAME,

40mg/kg/day, n=6); other groups received L-NAME plus indapamide (1mg/kg/day,n=6) or hydrochlorothiazide (10 mg/kg/day, n=6) or captopril (10 mg/kg/day, n=6) or combination of indapamide and captopril (n=6). The antihypertensives investigated were dissolved in the drinking water and administered orally for 7 weeks. At the end of the treatment the body weight (BW) and left ventricular weight (LVW) was measured and LVW to BW ratio was calculated (LVW/BW).

In the acute treatment, the bath medium was supplemented with the antihypertensives investigated (individually except the indapamide+captopril combination) in the same concentration 10^{-4} mol/l and the analysis of vasorelaxation was performed on femoral artery of normotensive Wistar rats and L-NAME hypertensive rats.

Blood pressure measurement

The blood pressure (BP) was measured non-invasively by the tail-cuff plethysmography using the Statham Pressure Transducer P23XL (Hugo Sachs, Germany) each weak. The final value was calculated from five successive measurements.

In vitro assessment of acetylcholine-induced relaxation by wire myograph

The endothelium-dependent relaxations were tested on femoral artery ring (approximately 1 mm long) using the Mulvany-Helper small vessel myograph (Dual Wire Myograph System 410A, DMT A/S, Aarhus, Denmark) under the isometric conditions. During the whole experiment the bath medium - Krebs-Ringer solution (containing in mmol/l: NaCl 118, KCl 5, $NaHCO_3$ 25, $MgSO_4.H_2O$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, EDTA

0.03, ascorbic acid 1.1, glucose 11), pH 7.4 was oxygenated (mixture of 95% O_2 and 5% CO_2) and kept at 37 °C. All the chemicals used were purchased from Sigma Chemicals Co. (Germany). In the chronic experiment the vessels were preconstricted with serotonin (10^{-5} mol/l). When the contraction reached a steady state the acetylcholine was added in cumulative manner (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} mol/l). In the acute experiment before the performance of preconstriction and relaxation the vessels were incubated with the substances investigated for 30 min in the same concentration 10^{-4} mol/l. The relaxations were expressed as a percentage of serotonin-induced contraction. Averaged value of vasorelaxation was calculated as a mean value of vasorelaxation reached in the groups based on the individual dose-response curves.

Statistical analysis

The results are expressed as means ± SEM. Significance of the differences between groups was determined by multifactorial analysis of variance (ANOVA) followed by Bonferroni post-hoc test. Probability values less than 0.05 were considered to be significant.

Results

Blood pressure

Blood pressure of control rats as well as rats receiving L-NAME and L-NAME simultaneously with indapamide, hydrochlorothiazide, captopril, and indapamide+captopril combination is expressed in the figure 1. Captopril and indapamide+captopril significantly decreased blood pressure rise from the 2nd week of

treatment in comparison with the group receiving L-NAME only. Both diuretics – indapamide and hydrochlorothiazide were able to decrease blood pressure rise from the 3rd week of treatment (Fig. 1).

Body weight, left ventricular weight and LVW/BW ratio

The body weight was not influenced by L-NAME alone or plus indapamide, hydrochlorothiazide, and captopril treatment. The indapamide+captopril combination decreased BW significantly. Left ventricular weight was increased significantly in rats receiving L-NAME. The antihypertensive treatment decreased left ventricular weight in comparison to the L-NAME group. LVW/BW was increased in L-NAME hypertensive rats in comparison with the control Wistar rats. This augmentation was prevented by concomitant treatment with all antihypertensives investigated (Tab. 1).

In vitro assessment of acetylcholine-induced relaxation by wire myograph

Chronic experiment

Chronic L-NAME treatment did not decrease the acetylcholine-induced relaxations of femoral artery. We have recorded a significant increase of averaged acetylcholine-induced relaxations only after chronic indapamide+captopril combination treatment. This increase was significant comparing to both control and L-NAME treated groups (Fig. 2).

Acute experiments

Incubation of femoral artery isolated from L-NAME-hypertensive rats with captopril and indapamide+captopril improved significantly the averaged acetylcholine-induced

relaxation compared to non-incubated vessels from L-NAME-hypertensive rats. Indapamide+captopril incubation manifested also significant improvement of relaxations in comparison to femoral artery incubated with indapamide alone (Fig. 3). After indapamide, hydrochlorothiazide, captopril, and indapamide+captopril incubation of femoral artery isolated from Wistar control rats we have recorded significant augmentation of averaged acetylcholine-induced relaxations in comparison to non-incubated vessels from Wistar control rats (Fig. 4).

Discussion

This study demonstrates that antihypertensive (indapamide, hydrochlorothiazide, captopril, and indapamide+captopril combination) treatment prevented blood pressure increase and left ventricular hypertrophy development induced by L-NAME. At the end of experiment, there was no significant difference between the blood pressure of rats receiving L-NAME simultaneously with antihypertensives and normotensive Wistar rats.

Interestingly, in our experiment, chronic L-NAME treatment did not decrease the acetylcholine-induced relaxation of femoral artery. Indapamide+captopril combination along with L-NAME was able to increase relaxation responses of femoral artery above the control level. Incubation of femoral artery isolated from L-NAME-hypertensive rats with captopril and indapamide+captopril improved significantly acetylcholine-induced relaxations compared to non-incubated vessels from L-NAME-hypertensive rats. Finally, all the antihypertensives investigated were able to increase relaxation responses of femoral artery isolated from normotensive Wistar rats.

The new group of thiazide-like diuretics, including indapamide, possess minimal diuretic but significant antihypertensive effect. The probable mechanism of their action include the restoration of electrolyte balance, diminished responses to vasoconstrictor agents and reduced peripheral resistance (Levy *et al.* 1990, Szilvassy *et al.* 2001). In agreement with our study, indapamide significantly reduced the blood pressure in DOCA-salt sensitive rats which was accompanied by the decrease of peripheral resistance (Levy *et al.* 1990). In *in vitro* experiments Boulanger *et al.* (1993) recorded that indapamide inhibited endothelium-dependent vasoconstriction of aorta from spontaneously hypertensive rats. In our acute experiments, indapamide was not able to increase relaxation of femoral artery isolated from L-NAME hypertensive rats. On the other hand, it increased relaxation of femoral artery isolated from control Wistar rats. In human brachial arteries Pickkers *et al.* (1998) documented the direct vasodilative effect of hydrochlorothiazide which was associated with activation of potassium channels. In our experimental conditions, hydrochlorothiazide, similarly like indapamide, had no effect on the relaxation responses of femoral artery isolated from L-NAME hypertensive rats both after chronic treatment and acute incubation. However, after acute incubation both diuretics increased relaxation responses of femoral artery isolated from control Wistar rats. Thus, we hypothesised that nitric oxide may be involved in the improvement of vasorelaxation induced by indapamide and hydrochlorothiazide.

Beside direct reduction of angiotensin II and elevation of bradykinin production, the increase of NO generation and decrease of reactive oxygen species (ROS) formation is probably responsible for beneficial effects of ACE inhibitors (Wiemer *et al.* 1997, Pecháňová *et al.* 1997, 2006, 2007, Šimko *et al*. 1999, 2001, Gvozdjáková *et al.* 2001). The presence of the thiol group in the captopril molecule contributes to its strong

antioxidative potential (Török *et al.* 2002, Zicha *et al.* 2006b, Pecháňová *et al.* 2006, 2007). Bernátová *et al.* (1996) demonstrated that captopril in the dose 100mg/kg/day prevented L-NAME-induced hypertension and left ventricular hypertrophy without affecting NO synthase activity. In accordance with this finding, in this experiment chronic captopril administration in ten time lower dose (10 mg/kg/day) prevented the blood pressure rise due to the L-NAME treatment, however, without affecting endothelium-dependent relaxation of femoral artery. On the other hand, Dahlof and Hansson (1993) showed that enalapril reduced blood pressure via dilatation and decreased peripheral resistance in men with previously non-treated essential hypertension. Also Keaton *et al.* (1998) demonstrated that chronic captopril treatment improved vasorelaxation of spontaneously hypertensive rats. Our findings confirmed the beneficial effect of captopril only in acute experiments: this ACE inhibitor increased the relaxation responses of femoral artery isolated from both L-NAME hypertensive and control Wistar rats. Thus, we hypothesized that besides NO, other substances such as prostacyclin and endothelium derived hyperpolarizing factor (EDHF) may be involved in the improvement of vasorelaxation after both acute captopril and chronic indapamide+captopril combination treatment. Interestingly, chronic captopril treatment did not improve vasorelaxation also in spontaneously hypertensive rats where the production of NO was not blocked (Sládková *et al.* 2005). We assume that the dose of captopril (10 mg/kg/day) used in the chronic experiment was not able to enhanced sufficiently the production of NO with subsequent vasorelaxation.

We showed that indapamide+captopril combination prevented most effectively the blood pressure elevation. Several studies pointed out the fact that the antihypertensive therapy combining drugs from different classes provide an additive effect and thus

minimize the possibility of adverse effects depending on the dose used (Toblli *et al.* 2003). Low-dose combination treatment with perindopril or captopril and indapamide had been shown to increase NO synthase activity and endothelium-dependent relaxation in spontaneously hypertensive and Dahl salt-sensitive rats (Hayakawa *et al.* 1997, Sládková *et al.* 2005, Kojšová *et al.* 2005, 2006). In this experiment, however, indapamide was not able to exceed the inhibitory effect of L-NAME and to enhance vasorelaxation.

In conclusion, our study demonstrated that indapamide+captopril combination along with L-NAME was able to increase relaxation responses of femoral artery above the control level. Acute incubation with captopril or indapamide+captopril improved relaxation responses of femoral artery from L-NAME hypertensive rats. Thus, nitric oxide is probably involved in indapamide-induced improvement of vasorelaxation while other vasorelaxing factors like prostacyclin or EDHF in the captopril-induced improvement of vasorelaxation. During the chronic treatment the additive and synergic effects of indapamide and captopril may contribute to the increase of vasorelaxation.

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model of cardiovascular disease and metabolic syndrome? *Physiol Res* **55**(Suppl 1):S49-63, 2006b.

Tab 1. The effect of L-NAME and L-NAME with indapamide, hydrochlorothiazide, captopril or indapamide + captopril combination on the body weight (BW), left ventricular weight (LVW) and left ventricular weight/body weight ratio (LVW/BW).

	n	BW [g]	LVW [mg]	LVW/BW [mg/g]
CT	6	341 ± 7	465 ± 29	1,36 ± 0,07
LN	6	349 ± 10	536 ± 20 $^{+}$	1,54 ± 0,06 $^{+}$
LN-I	6	357 ± 11	482 ± 14 *	1,36 ± 0,06*
LN-H	6	334 ± 15	441 ± 17 *	1,35 ± 0,08*
LN-C	6	339 ± 6	408 ± 4 *	1,21 ± 0,03 *
LN-I+C	6	305 ± 16 *	405 ± 5 *	1,29 ± 0,05 *

Wistar control rats (CT); rats treated with L-NAME 40 mg/kg/day (LN), rats treated with L-NAME plus indapamide 1 mg/kg/day (LN-I), hydrochlorothiazide 10 mg/kg/day (LN-H), captopril 10 mg/kg/day (LN-C), indapamide-+captopril combination 1 mg/kg/day+10mg/kg/day (LN-I+C). Results are shown as average ± SEM. $^{+}$p<0,05 *vs*. CT. *p<0,05 *vs*. LN.

Figure captions

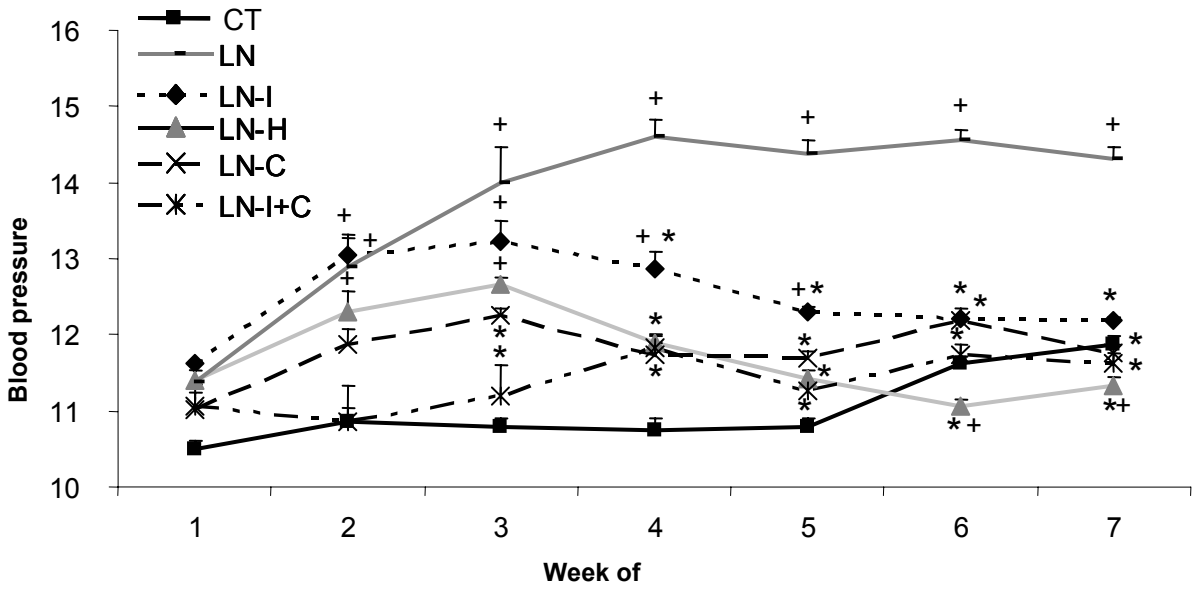
Figure 1 The effect of L-NAME and L-NAME with indapamide, hydrochlorothiazide, captopril or indapamide+captopril combination on the development of blood pressure. Wistar control rats (CT); rats treated with L-NAME 40 mg/kg/day (LN), rats treated with L-NAME plus indapamide 1 mg/kg/day (LN-I), hydrochlorothiazide 10 mg/kg/day (LN-H), captopril 10 mg/kg/day (LN-C), indapamide+captopril combination 1 mg/kg/day+10mg/kg/day (LN-I+C). Results are shown as mean ± SEM. +p<0,05 *vs*. CT, *p<0,05 *vs*. LN.

Figure 2 Averaged acetylcholine-induced relaxations of *arteria femoralis* isolated from rats treated with L-NAME or L-NAME with indapamide, hydrochlorothiazide, captopril or indapamide+captopril combination. Wistar control rats (CT); rats treated with L-NAME 40 mg/kg/day (LN), rats treated with L-NAME plus indapamide 1 mg/kg/day (LN-I), hydrochlorothiazide 10 mg/kg/day (LN-H), captopril 10 mg/kg/day (LN-C), and indapamide+captopril combination 1 mg/kg/day+10mg/kg/day (LN-I+C). Results are shown as average ± SEM. +p<0,05 *vs*. CT, *p<0,05 *vs*. LN.

Figure 3 Averaged acetylcholine-induced relaxations of *arteria femoralis* isolated from L-NAME-hypertensive rats and incubated with indapamide, hydrochlorothiazide, captopril or indapamide+captopril. Non-incubated vessels from L-NAME-hypertensive rats (LN); vessels incubated in the same concentration 10^{-4} mol/l with indapamide (I), hydrochlorothiazide (H), captopril (C), indapamide+captopril combination (I+C). Results are shown as average ± SEM. *p<0,05 *vs*. LN, **#**p<0,05 I *vs.* I+C.

Figure 4 Averaged acetylcholine-induced relaxations of *arteria femoralis* isolated from control Wistar rats and incubated with indapamide, hydrochlorothiazide, captopril and indapamide+ captopril combination. Non-incubated vessels (CT); vessels incubated in the same concentration 10^{-4} mol/l with indapamide (I), hydrochlorothiazide (H), captopril (C), indapamide+captopril combination (I+C). Results are shown as average ± SEM. *p<0,05 *vs*. CT**.**

Figure 1


CT
LN
LN-I
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LN-I+C
Blood pressure
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Figure 2

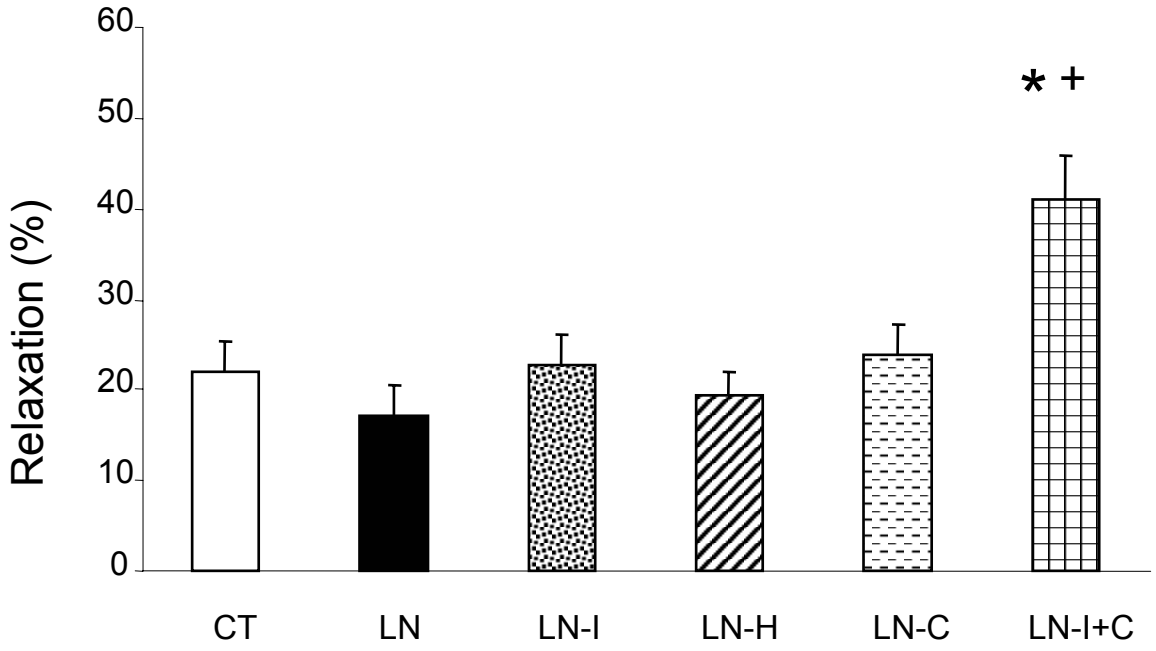

Figure 3

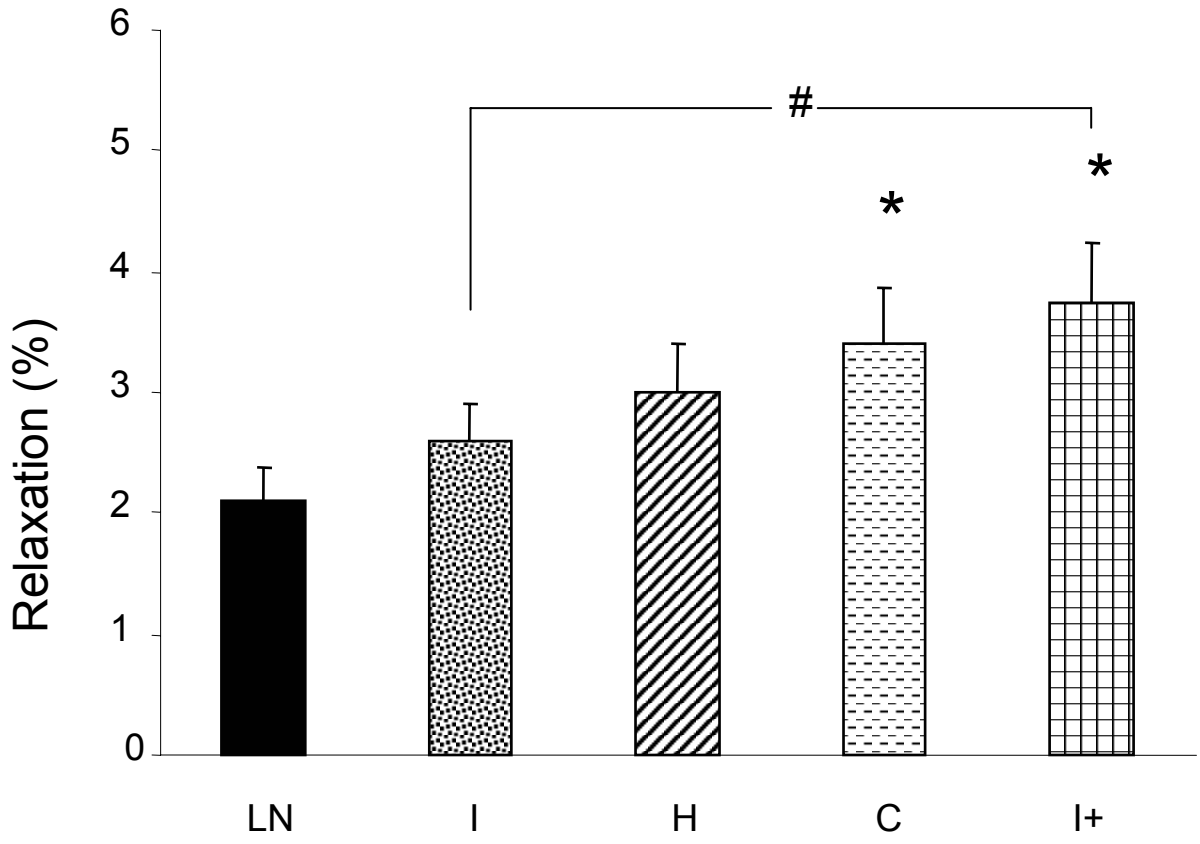

Figure 4