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Carbon monoxide does not contribute to vascular tonus improvement in exercise-trained rats with chronic nitric oxide synthase inhibition

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ABSTRACT

Carbon monoxide (CO), an end product of heme oxygenase (HO) that is involved in the regulation of vascular tonus, may show a compensatory effect in nitric oxide (NO) deficiency. This study aimed to assess the effect of the HO/CO system on the vascular tone in exercise-trained rats with hypertension induced by chronic NO synthase (NOS) inhibition. Hypertension was induced by *N*-nitro-L-arginine methyl ester (25 mg/kg/day in drinking water), and exercise training comprised swimming 1 h/day, 5 days/week, for 6 weeks. Systolic blood pressure (BP) was measured weekly using a tail-cuff method. The effects of hypertension and/or exercise-training on the constriction and relaxation responses of the thoracic aorta and resistance arteries of the mesenteric and gastrocnemius vascular beds were evaluated. NOS inhibition produced a gradually developed hypertension, and the magnitude of the increase in BP was significantly attenuated by exercise training. Although phenylephrine (Phe)-induced contraction responses of aorta incubated with an HO-1 inhibitor were reduced in hypertensive animals, there was no difference in the hypertensive-exercise group. However, thoracic aortas in the hypertensive-exercise group exhibited significantly more relaxation in response to a CO donor. There was no change in Phe-induced contraction with or without HO inhibition CO donor relaxation responses in both resistance arteries. These results suggest that the HO/CO system does not contribute to diminishing BP by exercise training in a NOS inhibition-induced hypertension model.

1. Introduction

Carbon monoxide (CO) is a gaseous molecule endogenously produced during the degradation of heme into iron and biliverdin catalysed by heme oxygenase (HO) [1]. Endogenous CO and exogenously applied CO have been shown to induce vasorelaxation in different vascular tissues from different animal species [2]. The HO/CO system plays an important role in the regulation of vascular tone and blood pressure [3,4]. Systemic administration of HO inhibitors has been reported to increase blood pressure in rats [5]. On the other hand, the pharmacological induction of HO-1 or the administration of HO substrates, which results in increased CO production, also normalises blood pressure in spontaneously hypertensive rats (SHR) [6–8] and several hypertension models [9–11]. Furthermore, vascular HO-1 expression increases in SHR and several experimental models of hypertension (angiotensin II-induced hypertension, Dahl-Rapp salt-sensitive rats and deoxycorticosterone salt-sensitive rats) [12]. These observations indicate that the upregulation of the HO/CO system in these hypertensive models is believed to serve as a protective compensatory mechanism against hypertension. It was also shown that HO mRNA levels in the left ventricle, aorta, kidney and soleus muscle were elevated in a nitric oxide synthase (NOS) inhibition-induced hypertension model [13]. Both CO and NO can modulate the formation and actions of each other, and there is cross-talk between the HO/CO and nitric NOS/NO systems [14–16]. Lower concentrations of CO may induce the release of NO from a large intracellular pool and therefore may mimic the vascular effects of NO [17].

It has been known for many years that regular physical activity has a blood pressure-lowering effect in humans [18]. This beneficial

Abbreviations: NO, Nitric oxide; CO, carbon monoxide; HO, heme oxygenase; NOS, nitric oxide synthase; SHR, hypertensive rats; L-NAME, *N*-nitro-L-arginine methyl ester; sGC, soluble guanylate cyclase enzyme; Phe, phenylephrine; Ach, acetylcholine; CrMP, chromium mesoporphyrin; CORM, carbon monoxide-releasing molecule; ODQ, 1H-[1,2,4]Oxadiazolo[4,3-a] quinox-alin-1-one; TEA, tetrahylammonium chloride; CS, Citrate synthase.

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effect of exercise has also been demonstrated in various experimental animal models of hypertension [19–21]. Our previous studies first showed that exercise training significantly reduces systolic blood pressure in hypertensive rats with chronic NOS inhibition [20,22,23]. Regarding the mechanism through which exercise lowers blood pressure in hypertension induced by NOS inhibition, the results of our studies prove it to be an increase in NOS activity of skeletal muscle and positive structural changes in the vessel wall [20]. We also demonstrate that increased endothelial NOS expression and vasorelaxation responses were improved in response to chemical stimulation by acetylcholine and physical stimulation by shear stress in resistance arteries of exercise-trained rats with chronic NOS inhibition [23]. It could be difficult to explain these results by considering only the increase in endothelial NOS expression owing to the improvement of vessel responses in hypertension induced by NOS inhibition in the presence of L-NAME (*N*-nitro-L-arginine methyl ester) and likely to be the contribution of another endogenous vasodilator.

Sun et al. indicated that acute and chronic endurance exercise was associated with not only elevated endothelial NOS but also elevated vascular HO-1/HO-2 and enhanced HO-related vasodilation [24]. Training exercise has been reported to increase vasodilatation in response to exogenous CO in Goldblatt hypertensive rats [25]. However, the effect of the HO/CO system on vasoreactivity in exercise-trained rats with chronic NOS inhibition is not yet known.

Given the above-mentioned findings, the purpose of the present study was to investigate role of HO-derived CO on the regulation of vascular tone and expression of HO isoforms in swimming exercise-trained rats with hypertension induced by chronic NOS inhibition. The effect of exercise training on rats with NOS-inhibited hypertension was investigated in both conduit and resistance arteries. We hypothesised that regular swimming exercise causes an endogenous CO contribution to vascular tonus in NOS-inhibited hypertensive rats.

2. Materials and methods

2.1. Animals and design

Forty adult male Wistar rats (6–9 months old) were used in the present study. The animals were housed at 23 ± 2 °C on a 12:12-h light–dark cycle and had unrestricted standard rat chow and drinking water. Rats were assigned randomly to four different groups: sedentary control (C; n = 10), exercise training (E; n = 10), sedentary hypertensive (H; n = 10) and hypertensive-exercise (HE; n = 10). In two of the groups (H and HE), hypertension was induced by oral administration of the NOS inhibitor L-NAME (25 mg/kg/day) dissolved in drinking water for 6 weeks. L-NAME was concomitantly given during the training protocol in the HE group. All other animals had access to normal tap water throughout the experiment (C and E groups). The animals in the training groups were subjected to swimming exercise (60 min/day, 5 days/week for 6 weeks) in a glass tank (100 × 50 × 50 cm deep) filled with tap water (32–34 °C). The duration of the first swimming experiment was limited to 10 min and increased by 10 min daily up to 60 min. The experimental protocol was approved by the Animal Care and Use Committee of Akdeniz University and followed the guidelines for the use of animals in experimental research.

The systolic blood pressure of rats was measured using a non-invasive tail-cuff method. Measurements were made at the beginning of the study and every 2 weeks during for 6 weeks. Data were obtained using a MAY-BPHR 9610-PC unit and MP 150 data-acquisition system (BIOPAC Systems, Santa Barbara, CA, USA). In exercising animals, the final measurements were performed 1 day after the last swimming session. Two different vessel beds were used as resistance arteries in this study: the gastrocnemius muscle artery, which is affected by exercise training, and one of the mesenteric arteries, which is not affected by exercise training. The thoracic aorta was also used as conduit artery.

2.2. Preparation of vessel

The rats were killed under pentobarbital anaesthesia by withdrawing blood from the abdominal aorta. The gastrocnemius muscle, mesenteric bed and thoracic aorta were quickly removed and transferred to a dissecting dish filled with cold Krebs solution containing (in mM) 110 NaCl, 5 KCl, 24 $NaHCO_3$, 1 KH_2PO_4, 1 $MgSO_4$, 2.5 $CaCl_2$, 0.02 EDTA and 10 glucose. Thoracic aortas were isolated and cut into 2- to 3-mm-long rings and mounted in an organ bath containing 20-mL Krebs solution and connected to an isometric force transducer (FDT 10-A; MAY, Ankara, Turkey). The solution was maintained at pH 7.4 gassed with 95% O_2 and 5% CO_2 at 37 °C. The rings were stretched to a basal tension of 1 g. The first-order (1A) branches of the gastrocnemius feed artery (200–240 μm in diameter) and the second-order (2A) branches of the mesenteric artery (200–220 μm in diameter) were isolated under a dissection microscope and dissected into segments of 2 mm in length. Vessel segments were then put into a wire myograph (EMKA Technologies, Paris, France). Basal wall tensions of vessel segments were calculated using computer software (Normalize version 1.0; EMKA Technologies). All vessels were rested at the determined baseline for 1 h at 37 °C.

2.3. Protocols

After an equilibration period, the endothelium was examined following vitalisation using 20-mM KCl $+10^{-7}$-M phenylephrine (Phe) (Sigma, St. Louis, MO, USA), which is a precondition for vessels. The presence of functional endothelium was assessed in all rings by the ability of 10^{-6} M acetylcholine (Sigma) to induce >70% relaxation of vessels pre-contracted with submaximal Phe (10^{-6} M). Each of the following stages of the experimental protocol was evaluated in the presence of the non-specific NOS inhibitor L-NAME (1 mM; Sigma) in the bath solution. NO production was inhibited in CO studies to observe the real effect of CO. Resting intervals of 30 min were given between the protocols.

Endogenous CO responses. Contraction responses to Phe (10^{-9} to 3×10^{-5} M) were recorded cumulatively. Following a 30-min incubation of vessels with the HO inhibitor chromium mesoporphyrin (CrMP) (30 μM; Frontier, Utah, USA), Phe dose–responses curves were recorded again. After suppressing the CO production by CrMP incubation, the expected increase in the Phe-induced contraction response was used to demonstrate the contribution of endogenous CO to vascular tonus.

Exogenous CO responses. Vessels were pre-contracted with 3×10^{-6} M Phe, and then the vasodilation responses to the cumulative doses (10^{-9} to 3×10^{-4} M) of tricarbonyldichlororuthenium (II) dimer acting as a CO-releasing molecule (CORM) (Sigma-Aldrich, St. Louis, MO, USA) were recorded [26,27].

2.4. Determination of heme oxygenase isoforms' expression

To determine HO isoform expression, arterial tissues were stored at −80 °C, and 100 μg of protein lysates were applied per line, separated by SDS-PAGE (in 12.5% gel) and transferred onto polyvinylidene difluoride membranes by electroblotting. Membranes were blocked in 5% non-fat dry milk and then incubated overnight at 4 °C with primary antibodies against HO-1 (1:1000; StressGen, Victoria, BC, Canada), HO-2 (1:1000; StressGen) and β-actin (1:500; Abcam, Cambridge, UK). The membranes were then