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Carbon monoxide contributes to the regulation of vascular tonus in renal resistance arteries in spontaneously hypertensive rats

Karbon monoksit, spontan hipertansif sıçanların renal direnç arterlerinde vasküler tonusun düzenlenmesine katkıda bulunur

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ABSTRACT

Objective: Carbon monoxide (CO), an end product of heme oxygenase (HO) involved in the regulation of vascular tone, may show a compensatory effect in the course of hypertension. This study aimed to assess the effects of the HO/CO system on the vascular tone of renal resistance arteries in spontaneously hypertensive rats (SHRs).

Methods: The contribution of endogenous CO to vascular tone in renal resistance arteries was evaluated by the phenylephrine (Phe) contraction response with or without an HO inhibitor. The effect of the exogenous CO relaxation response was assessed by a CO releasing molecule (CORM). The mechanism of the CO relaxation effect was evaluated by a guanylate cyclase inhibitor, ODQ, or a potassium channel blocker, TEA. HO-1 and HO-2 enzyme protein expressions in renal resistance arteries were determined by Western blot analysis.

Results: Phe-induced constriction responses were higher in renal resistance arteries of SHRs compared to control animals. The extent of the same type of constriction was even higher in the SHR group after inhibition of CO production. Relaxation responses to a CO donor, CORM, were greater in the SHR group versus the control group. TEA, but not ODQ, suppressed CORM

ÖZET

Amaç: Vasküler tonusun düzenlenmesinde yer alan karbon monoksit (CO), hipertansiyon gelişimi sırasında kompansatuar etki gösterebilir. Bu çalışma, spontan hipertansif sıçanlarda (SHR) HO / CO sisteminin renal direnç arterlerinin vasküler tonusu üzerine etkilerini değerlendirmeyi amaçlamıştır.

Yöntem: Renal direnç arterlerinde endojen CO'nun vasküler tonusa katkısı, bir HO inhibitörü varlığında ve yokluğunda fenilefrin (Phe) kontraksiyon yanıtı ile test edildi. Ekzojen CO gevşeme yanıtı da CO donörü (Carbon monoxide-releasing molecule (CORM)) kullanılarak değerlendirildi. CO gevşeme yanıtının mekanizması, guanilat siklaz inhibitörü (ODQ) veya potasyum kanal blokörü TEA ile değerlendirildi. Renal direnç arterlerindeki HO-1 ve HO-2 protein ekspresyonları Western blot analizi ile saptandı.

Bulgular: SHR'lerde kontrol grubuna kıyasla Phe'ye verilen kasılma yanıtı anlamı olarak yüksekti ($p<0,05$). HO inhibisyonu ile CO üretimi engellendiğinde SHR'de kasılma yanıtı daha da arttı ($p<0,05$). SHR sıçanlarda CORM'a verilen gevşeme yanıtları da daha yüksekti ($p<0,05$). Her iki grupta da CORM yanıtlarını ODQ baskılamazken TEA anlamlı

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responses in both groups. HO-2 protein expression patterns were not different between the groups, while HO-1 expression was remarkably higher in SHRs when compared to that in control rats.

Conclusion: Consequently, our results revealed a CO-based compensatory effect in SHRs by the induction of CO production and an increase in its bioavailability.

Key Words: CO, HO-1, SHR, renal resistance artery

olarak baskıladı ($p<0,01$). HO-2 protein ekspresyonu açısından gruplar arasında farklı olmamakla birlikte HO-1 ekspresyonu SHR'lerde kontrol grubuna göre önemli ölçüde daha yüksekti.

Sonuç: Sonuç olarak verilerimiz, SHR'lerin böbrek direnç arterlerinde karbon monoksidin kompansatuar etkisi olduğunu ortaya koymaktadır.

Anahtar Kelimeler: CO, HO-1, SHR, renal direnç arteri

INTRODUCTION

Carbon monoxide (CO) is one of the recently accentuated relaxing mediators contributing to vascular tone. CO is endogenously produced by the heme oxygenase (HO) enzyme via degradation of heme substances and acts as an important regulator of vascular tone and blood pressure (1, 2). Its vasodilator effect and consequent decrease in peripheral vascular resistance is based on the interaction with soluble guanylate-cyclase (sGC) or with the calcium-activated potassium channels located in vascular smooth muscle cells (2-4). CO and nitric oxide (NO) have similar physiological implications, and there is cross-talk between the two, which affects the production and function of each other (5). Deterioration of NO metabolism and function is a well-known process in the development of spontaneously hypertensive rats (SHRs). Accordingly, the expressions of HO-1 and HO-2 enzymes, which are endogenous sources of CO production, are upregulated during the course of hypertension to compensate for NO deficiency (5). Despite similar biological effects, CO and NO cannot replace each other. In some instances, their functions may be different; in others, their functions may be synergistic or antagonistic (6). Low CO concentrations induce NO release by eNOS activation, while high levels of CO reduce eNOS enzyme activity (7). The effects of the HO/CO system on renal vascular tone are well described. HO-1 and HO-2 expressions are present in both vascular and tubular structures of the kidney (8). CO acts as a vasodilator in the renal circulation regardless of being endogenous or exogenous (3, 4, 8, 9), and in vivo studies have demonstrated that the inhibition of HO enzymes reduces renal blood flow and depresses renal function (8, 10-12).

CO, produced during the development of hypertension, has protective effects on the kidney. For instance, besides its blood pressure-lowering effects by the stimulation of the HO-1 enzyme, shown in the one kidney - one clip (1K1C) renovascular hypertension model (13), and the angiotensin II (Ang II) salt-sensitive hypertension model stimulating renal damage (14), CO can also reduce renal damage. However, alterations in the HO/CO system in SHR kidneys are still unknown.

The kidney has important implications in blood pressure regulation, and renal damage by hypertension is common (15-17). Functional impairment of the renal vascular bed is an early sign of renal damage in both SHRs and other experimental

hypertension models. Renal vascular damage is first seen in the juxtamedullar cortex (18, 19), followed by an elevation in systemic blood pressure (20).

Hypertension leads to vascular damage of resistance vessels in the periphery, but renal vasculature is affected as well (20). There are no data elucidating the process, possible alterations in CO production, and CO bioavailability in renal resistance arteries of SHRs. It is speculated that CO acts as a backup for the NO molecule, and thus, the changes in the HO/CO system in resistance vessels of SHRs are worth investigating. Our present study aims to investigate if CO affects renal resistance arteries of SHR functionally, and if so, to evaluate the possible mechanisms involved in the process.

MATERIAL and METHOD

Animals

Animals in the hypertensive group were 12-15 week-old SHRs, while Wistar Kyoto rats (WKY) represented the age-matched normotensive group (Harlan Laboratories, Israel). The rats were housed on a 12:12 h light-dark cycle at 23 ± 2 °C with free access to standard rat chow and drinking water. All animal handling and experimental procedures were approved by the Animal Care and Usage Committee of Akdeniz University.

Systolic blood pressure measurements were performed using a noninvasive tail-cuff method. Blood pressure data were obtained with a MAY-BPHR 9610-PC unit and an MP 150 data-acquisition system (BIOPAC Systems; Santa Barbara, CA, USA) as measurements were completed immediately before euthanizing the animals.

Preparation of vessels

The animals were euthanized under pentobarbital anesthesia by withdrawing the blood from the abdominal aorta. Whole kidney samples were immediately removed and then transferred to a dissecting dish containing a cooled Krebs solution (110 mM NaCl, 5 mM KCl, 24 mM $NaHCO_3$, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 2.5 mM $CaCl_2$, 10 mM glucose, and 0.02 mM EDTA). Resistance arteries of renal tissues (the second-order; 2A branches of the renal artery; 200-220 µm in diameter) were carefully isolated and then dissected into 2-mm-long pieces under a dissection microscope. Vessel segments were then mounted onto a wire myograph chamber (EMKA Technologies, Paris, France), and their basal wall tensions were calculated using computer software (Normalize v1.0, EMKA Technologies, Paris, France). All vessel samples were rested for an hour at 37 °C and maintained at the determined baseline tension.

Protocols

The equilibration period was followed by a preconditioning and vitalization process by adding 20 mM KCl and 10^{-7} M phenylephrine (Phe, Sigma, St. Louis, USA)]. All vessel rings were then precontracted by a submaximal dose of Phe (10^{-6} M) and relaxation responses to 10^{-6} M acetylcholine (Sigma, St. Louis, USA) were evaluated to examine the presence of endothelium; >70% relaxation represented acceptable level. NO production was typically inhibited in CO studies to observe the true CO-based effects by the addition of a nonspecific NOS inhibitor L-NAME (1 mM, Sigma, St. Louis, USA) to the bath solution during each of the following steps in our experimental protocols. The chambers were left for 30 min resting intervals prior to the next protocol application, explained below.

Endogenous CO responses

Cumulative contraction responses to Phe (10^{-9} to 3×10^{-5} M) were recorded, and the vessels were then incubated with the HO inhibitor, chromium mesoporphyrin (CrMP; 30 µM, Frontier, Utah, USA) for 30 min to repeat the recording of Phe dose-response curves. The expected increase in the Phe-induced contraction response was used to demonstrate the contribution of endogenous CO to vascular tone after the suppression of CO production by CrMP treatment.

Exogenous CO responses

Cumulative doses (10^{-9} to 3×10^{-4} M) of tricarbonyldichlororuthenium (II) dimer (CORM, carbon monoxide-releasing molecule, Sigma-Aldrich, St. Louis, USA) were used to monitor vasodilation responses in vessel rings pretreated with 3×10^{-6} M Phe.

Determination of HO-1 and 2 expressions

The collected arterial tissue samples were stored at -80 °C before assessing the expression levels of HO isoforms. Then, 100 µg of tissue protein lysates were applied per lane for SDS-PAGE (in 12.5% gel) separation, followed by electroblotting to transfer the samples onto PVDF membranes. A 5% non-fat dry milk medium was used for the blocking process before the membranes were incubated overnight at 4 °C room temperature with primary antibodies: ß-actin (1:500; Abcam, Cambridge, UK), HO-1 (1:1000; StressGen, Victoria, BC, Canada), and HO-2 (1:1000; StressGen, Victoria, BC, Canada). All membranes were then treated for 1 h with appropriate HRP-conjugated secondary antibodies (1:80.000; Sigma, St. Louis, USA). The Image-J program was used for densitometric analysis and quantification of relative changes in protein expression.

Statistical analysis

The results are presented as mean ± SE. Non-repeated measurements (blood pressure, HO expression, and the maximum contraction/dilation responses-Emax) were evaluated by t-test (between groups) and paired t-test (within groups). Differences between the dose-response curves were tested for significance using two-way ANOVA for repeated measurements, followed by the Newman-Keuls post hoc test. A value of $p<0.05$ was considered statistically significant.

RESULTS

Mean arterial blood pressure levels were significantly higher in SHRs compared to the WKY control group ($p<0.001$, Fig. 1).

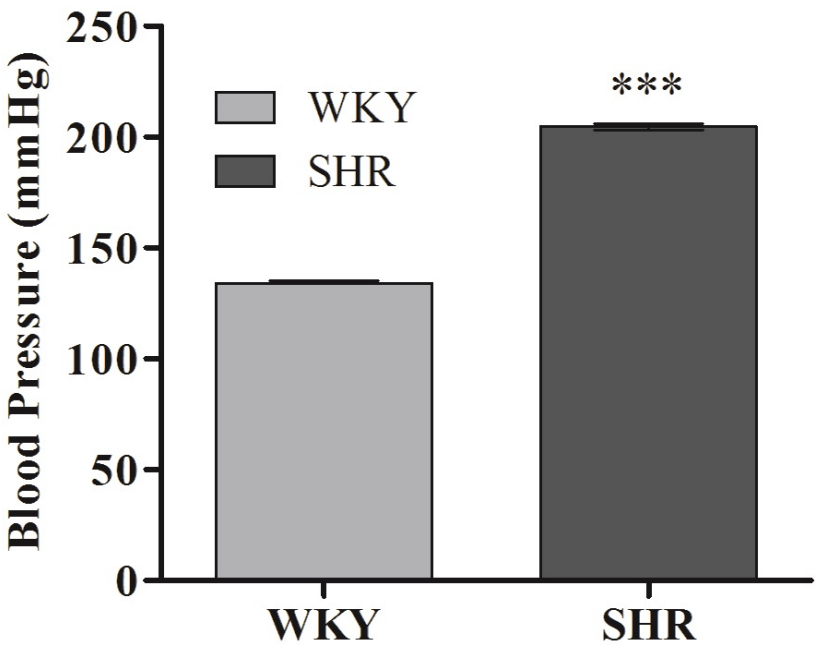

Figure 1. Systolic blood pressure (mmHg).
***p<0.001 difference from WKY

Endogenous CO responses

The differences between the contraction response to Phe and the endogenously produced CO response after pre-incubation with CrMP and exposure to Phe are shown in Fig. 2. Contraction dose-response curves to Phe were significantly higher in the SHR group compared to normotensive animals ($p<0.05$, Fig. 2A). When the HO inhibitor CrMP was used for pre-incubation prior to Phe application, no statistical change in contraction responses was seen in the WKY group, while a significant increase was observed in the SHR group ($p<0.05$, Fig. 2B). Comparison of Emax values of Phe constriction between the groups demonstrated significantly higher values in the SHR group than the WKY group ($p<0.05$, Fig. 2B). Comparison of Emax values of Phe contraction between samples with and without CrMP pre-incubation showed no difference in the normotensive group, but a statistically significant difference in the hypertensive group ($p<0.05$, Fig. 2B).

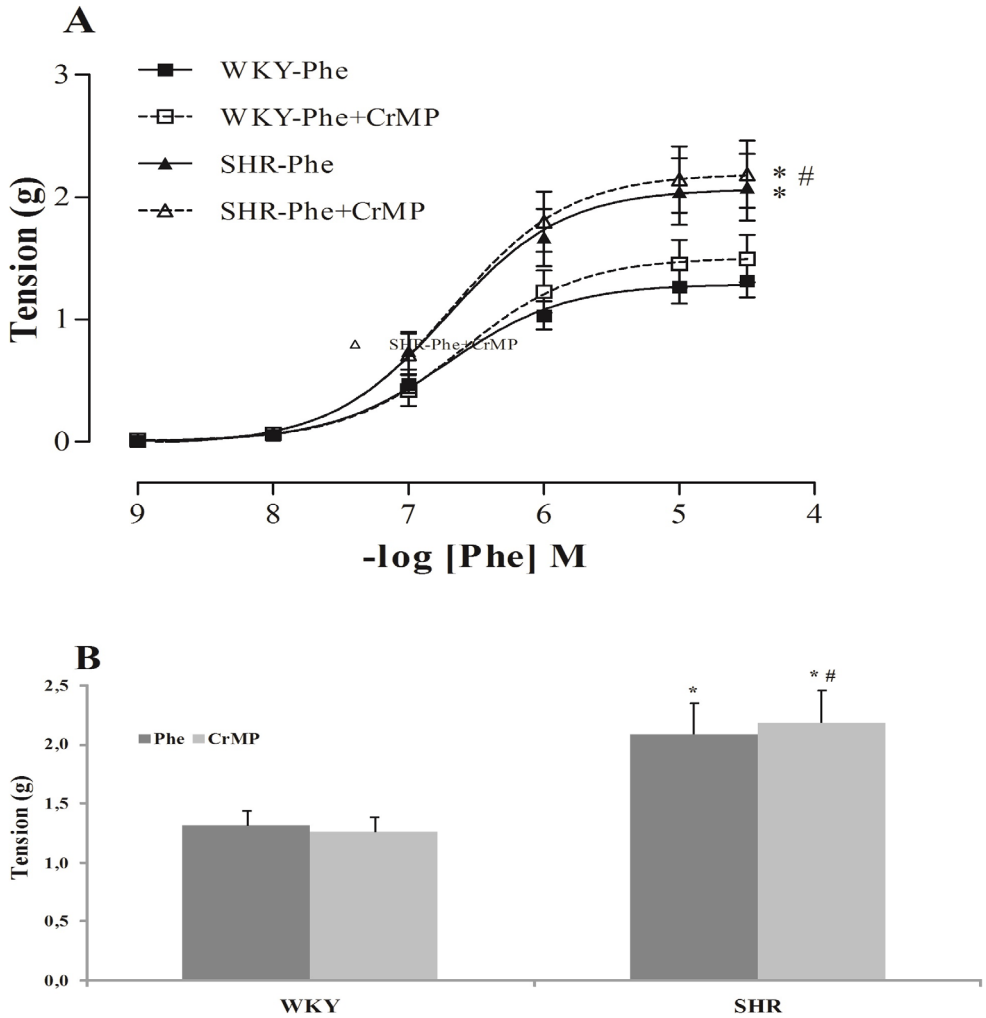

Figure 2. Constriction responses in renal resistance artery rings induced by Phe,
A; Dose-response curves to Phe at baseline and in the presence of HO inhibitor CrMP,
B; Emax values at baseline and in the presence of HO inhibitor CrMP.
* $p<0.05$ difference from WKY, # $p<0.05$ difference from basal Phe contractions for each group

Exogenous CO responses

Relaxation dose-response curves to the CO donor, CORM, as well as Emax values, were statistically higher in the SHR group compared to the WKY group ($p<0.01$, Fig. 3A and D). However, CORM responses after pre-incubation with ODQ or TEA were similar in WKY (Fig. 3B and D) and SHR (Fig. 3C-D) groups. CORM relaxation patterns were similar in both the groups with or without ODQ pretreatment, while TEA pre-incubation resulted in suppression of the CORM-induced relaxation response and Emax values in both groups ($p<0.01$).

HO-1 and 2 expressions

No differences in HO-2 protein expression was observed between the groups (Fig. 4B), but HO-1 expression was found to be higher in the SHR group compared to the normotensive control group ($p<0.01$, Fig. 4A).

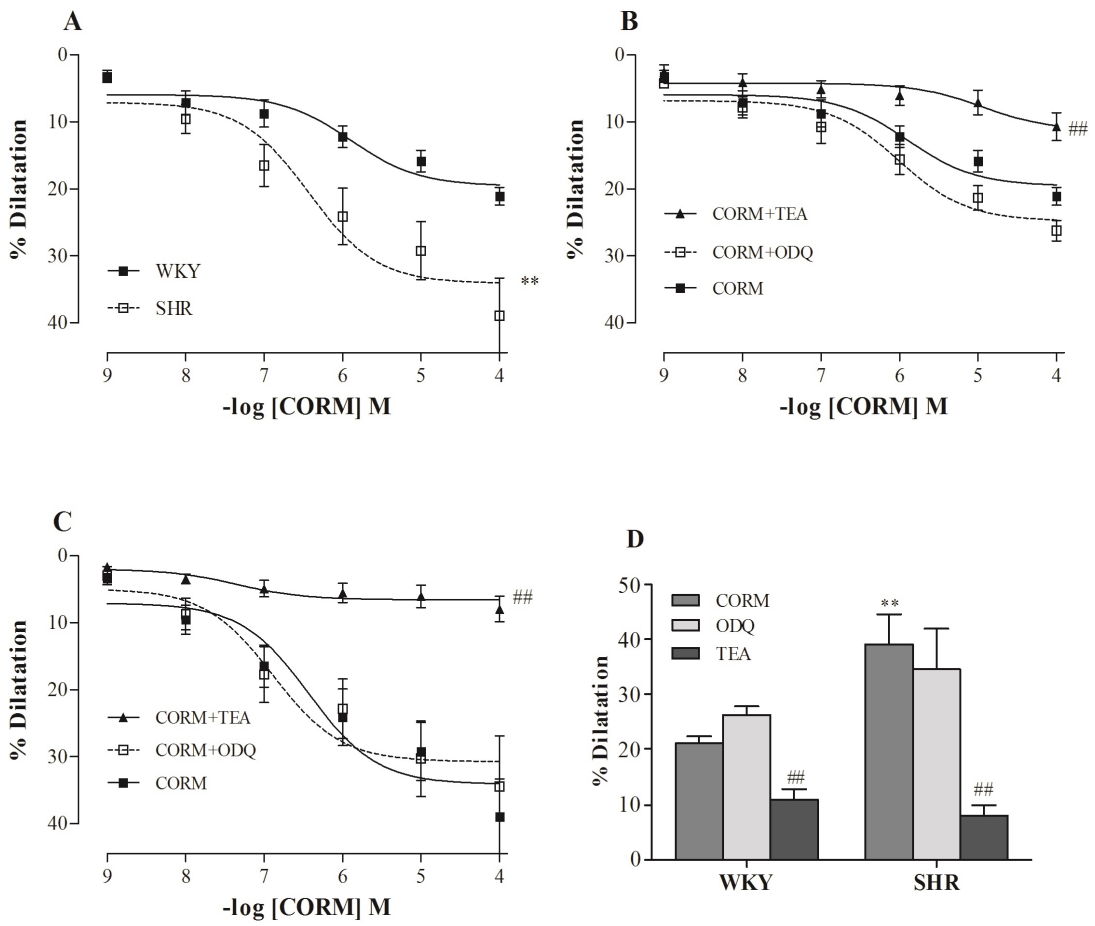

Figure 3. Dose-response curves in renal resistance artery rings for dilatation induced by CORM and maximal dilatation responses,
A; Curves representing CORM responses in both groups,
B; CORM responses in WKY at baseline and after pre-incubation with ODQ or TEA,
C; CORM responses in SHRs at baseline and after pre-incubation with ODQ or TEA,
D; Emax values at baseline and after pre-incubation with QDQ or TEA in experimental groups.
** $p<0.01$ difference from WKY, ## $p<0.01$ difference from basal CORM within groups

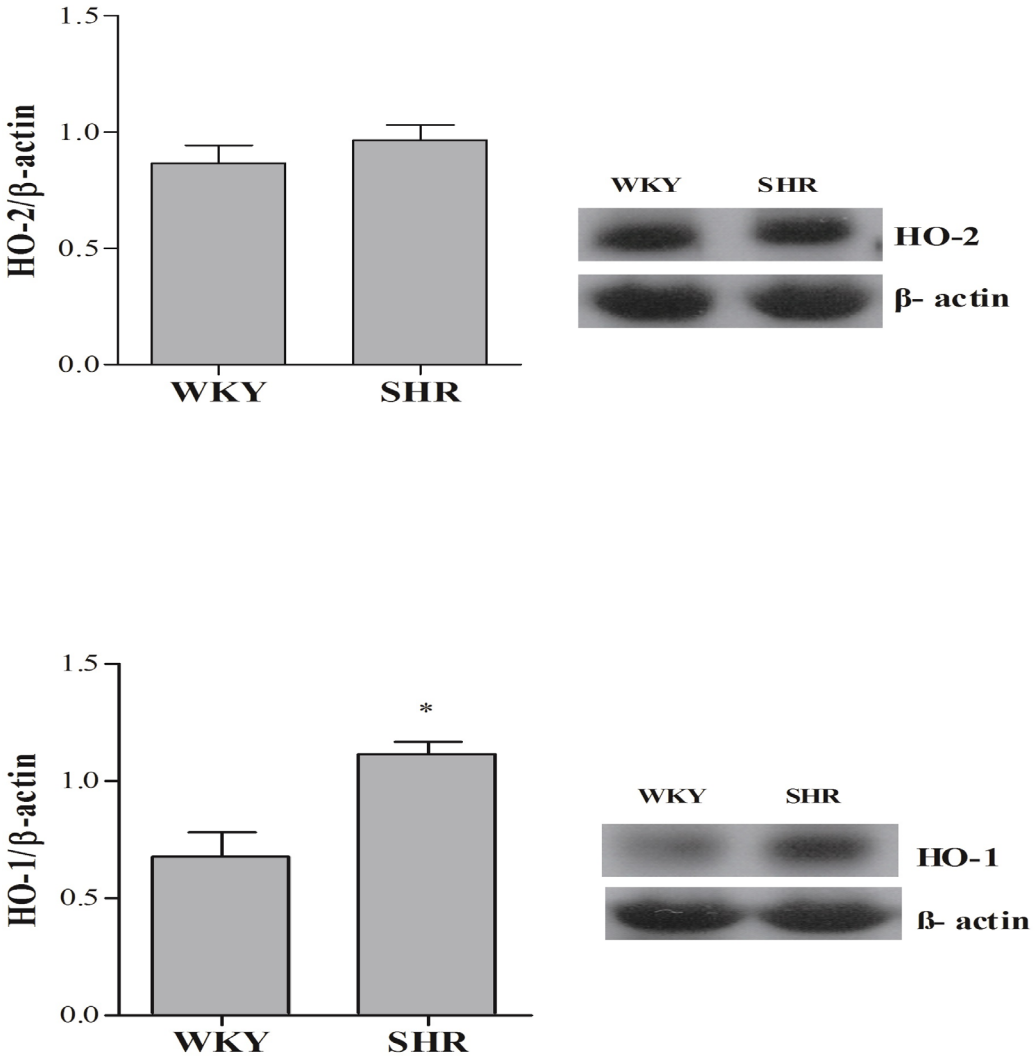

Figure 4. HO enzyme expression in renal resistance artery rings.
A; HO-1 expression,
B; HO-2 expression.
* p<0.05 difference from WKY

DISCUSSION

The kidneys are most the important organs to be affected by hypertension. This study investigated the contribution of the HO/CO system to vascular tone in renal resistance arteries in SHRs. Important findings of our study are; 1- The vasodilatory effect and endogenously contribution of CO increases because of the elevated HO-1 enzyme expression in renal resistance arteries in SHRs. 2-The vasodilatation response of CO primarily is induced by potassium channels in renal resistance arteries.

The contribution of CO to the regulation of blood pressure has been shown in various experimental hypertensive models (21). We used SHR, which is a model of essential hypertension in humans, in our study. As expected, the blood pressure values were found to be significantly higher in the SHR group

compared to the normotensive controls. In addition, greater vasoconstrictive responses in renal resistance arteries of SHRs reflect an increase in vascular tone. The increase in vasoconstrictive responses in SHRs induced by Phe is presented in other studies as well (21-23). However, the original finding of our study is that CO contributes to the increased vascular responses of renal resistance arteries in SHRs. Phe-induced constrictive responses in hypertensive animals, repeated after CRMP incubation (HO enzyme inhibitor), were seen in SHRs to a greater extent than in controls. The additional increase in the already elevated vascular tone in SHRs can be explained by the lack of compensatory effect of CO due to HO inhibition. However it is well known that the role of CO as a vasodilator and protective agent in renal circulation (2, 12, 24, 25) under physiological and some pathophysiological conditions, its protective effects against to elevated vascular tone in SHR was shown first time in this study.

The findings of Western blotting procedure for HO protein expression levels in renal resistance arteries support the hypothesis that CO has compensatory effects against the constrictive effects of Phe. Our expression results for the HO-2 enzyme, the constitutive form of HO, showed no differences between the groups, similar to the findings from other studies (26, 27). However, the expression of HO-1, the inducible form of HO, was significantly higher in the SHR group compared to the controls. Taken together, these results suggest that HO-1-derived CO contributes to the alleviation of the increased vascular tone in renal vascular tissues of SHRs. After the detection of elevated endogenous CO production in hypertensive animals, we proceeded to examine possible changes in responses to CO by evaluating the relaxation responses to CORM, a CO donor. Renal resistance arteries in the SHR group exhibited significantly increased dilation of 39% in SHR and 21% in WKY controls after CORM incubation.

CO exerts its vasodilator effect mainly by increasing the intracellular levels of cGMP (28) or by opening calcium-activated potassium channels (BKCa) in smooth muscles (29). The effects of CO on the relaxation mechanism were investigated by the inhibition of cGMP production and by the blockage of potassium channels by ODQ and TEA, respectively. Relaxation responses were not altered in either group after incubation with ODQ, but TEA incubation suppressed CORM-induced dilation by inhibiting potassium channels; a greater decline in relaxation responses was observed in the SHR group (39% to 7.9%) compared to the WKY group (21% to 10%). These data suggest a role of potassium channels in CO-dependent relaxation responses in renal resistance arteries of SHRs. These results are supported by previous studies, which revealed an increased conductance of potassium ions and BKCa density in aortas of SHRs (30, 31). A higher number of BKCa channels on the vascular smooth muscle surfaces may lead to improved vasodilation responses to exogenously applied CO.

The results of the present study indicate that CO production stimulated by an increase in HO-1 enzyme expression contributes to the suppression of vascular tone in renal resistance arteries. Relaxation responses to exogenous CO in renal resistance arteries were also improved. The compensatory effect of CO in renal resistance arteries of hypertensive animals is an important finding for delay in impairment in renal function.

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