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Different responses of mesenteric artery from normotensive and spontaneously hypertensive rats to nitric oxide and its redox congeners

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Abstract:
The conversion of nitric oxide ($NO^{\bullet}$) into its congeners nitrosonium (NO^{+}) and nitroxyl (HNO/NO^{-}) ions may have important consequences for signal transduction and physiological responses. Manganese-containing superoxide dismutase (MnSOD) may convert $NO^{\bullet}$ into its redox congeners. In our current work, we have examined the mechanism of sodium nitroprusside (SNP)-induced relaxation of arteries, with or without endothelium, from both normotensive and spontaneously hypertensive (SH) rats in the absence and presence of MnSOD. SNP induced a greater degree of relaxation in normotensive than in SH rats. MnSOD antagonized SNP-induced relaxation and effect was greater in normotensive than hypertensive rats. However, MnSOD even potentiated SNP-induced relaxation in mesenteric arteries with endothelium from SH rats. Our results indicate that HNO/NO^{-}-mediated relaxation is more effective in mesenteric artery smooth muscle from SH rats than from normotensive rats and that vascular dysfunction in SH rats is not solely endothelium-derived but involves changes in vascular smooth muscles.

Key words:
endothelium, hypertension, mesenteric artery, MnSOD, sodium nitroprusside

Abbreviations: Ach – acetylcholine, CGRP – calcitonin gene-related peptide, CuZnSOD – copper-zinc superoxide dismutase, FeSOD – iron-containing superoxide dismutase, HNO/NO^{-} – nitroxyl, MnSOD – manganese-containing superoxide dismutase, NO – nitric oxide, NO^{+} – nitrosonium, $O_2^{\bullet -}$ – superoxide anion radical, $ONOO^{-}$ – peroxynitrite, Ph – phenylephrine, ROS – reactive oxygen species, SH – spontaneously hypertensive, SNP – sodium nitroprusside

Introduction

Nitric oxide ($NO^{\bullet}$) synthesized in the vascular endothelium as a consequence of physiological, chemical and physical stimuli maintains the vasodilatory tone that is essential for keeping the normal blood flow and

arterial blood pressure, normal heart function, hemostasis and relaxation of vascular smooth muscle [16]. The vascular endothelium is an important metabolic and endocrine organ that has an important role in homeostasis, vasorelaxation and, in some situations, hypertension. Endothelial dysfunction may be due to classic risk factors and due to the presence of various diseases. In addition, the imbalance between production and/or degradation of $NO^{\bullet}$ and the superoxide anion radical ($O_2^{\bullet-}$) is a cause of increased vascular resistance. The interaction between $NO^{\bullet}$ and $O_2^{\bullet-}$ radicals, that results in the formation of vasculotoxic peroxynitrite ($ONOO^-$) and nitrosothiols, leads to destructive vicious cycles damaging the endothelium and vascular smooth muscle cells [7]. Oxidative stress alters many functions of the endothelium including modulation of vasomotor tone. Inactivation of $NO^{\bullet}$ by $O_2^{\bullet-}$ and other reactive oxygen species (ROS) occurs in such conditions as hypertension, hypercholesterolemia, diabetes and nicotine addition. Loss of $NO^{\bullet}$ associated with these traditional risk factors may in part explain why it predisposes to atherosclerosis [4].

The effect of SOD on the circulation and relaxation of smooth muscle has been studied [22, 24] from the point of view of SOD's influence on the concentration of $O_2^{\bullet-}$, blockage of $ONOO^-$ production and extending the half-life of $NO^{\bullet}$. MnSOD isolated from *E. coli* has been shown to possess the greatest anti-inflammatory activity in models, including adriamycin-induced edema [8] and adjuvant-induced polyarthritis [9] when compared with SODs isolated from other sources. A possible explanation for such a difference could be related to the fact that exposure of MnSOD and FeSOD but not Cu/ZnSOD to $NO^{\bullet}$ leads to nitrosonium (NO^+) and nitroxyl (HNO/NO^-) generation causing enzyme modification and inactivation [6, 19]. The physiological effects of HNO/NO^- are due to its interaction with endogenous neuropeptides such as calcitonin gene-related peptide (CGRP). This 37-amino acid peptide is the most potent vasodilator known to date and is thought to be involved in the regulation of resting blood pressure and regional blood flow [13]. Due to the direct connections between the biochemical and physiological actions of $NO^{\bullet}$, HNO/NO^- and NO^+, there are now new reasons to analyze the effects of $NO^{\bullet}$ donors on important physiological functions such as artery relaxation. Taking into account that CuZnSOD can react with NO^- to generate $NO^{\bullet}$ [17] and that $O_2^{\bullet-}$ [24] and hydrogen peroxide (H_2O_2) can have a direct effect on smooth muscle [14, 18], a complex network of possible actions of these enzymes in blood vessels can be established. Due to the important and discrete physiological roles of $NO^{\bullet}$, HNO/NO^- and NO^+, the analysis of the effects of SNP and MnSOD on arterial relaxation is an attractive topic worthy of study.

Materials and Methods

Artery preparations

Experiments were performed using mesenteric arteries isolated from male Wistar and SH rats (250–300 g, 6 months old). All protocols for handling the rats were approved by the local ethics committee for animal experimentation that strictly followed international regulations. The adhering perivascular tissue was carefully removed from arteries that were then cut into 3–5 mm ring segments and incubated for 30 min in Krebs-Ringer bicarbonate solution at 36°C continuously oxygenated with a mixture of 95% oxygen and 5% carbon dioxide. The rings were equilibrated for 30 min under 2 g of resting tension. An isometric transducer (Ugo Basile, 21025 Comerio, Italy) registered mechanical contractions. Contractions of isolated blood vessels were provoked by phenylephrine (10^{-6} M) (Sigma-Aldrich, Taufkirchen, Germany) and the functional integrity of the endothelium was confirmed by acetylcholine (10^{-5} M) (Serva, Feinbiochemica Heidelberg, Germany) and by histopathological examination. The latter indicated that the endothelium was better preserved in normotensive than in SH rats [14]. The percentage of relaxation caused by acetylcholine depended on the degree of endothelial preservation. In SH rats the endothelium is continuously damaged due to high blood pressure, therefore, the relaxing effect of acetylcholine is much lower than in normotensive rats [12].

Increasing concentrations (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) of sodium nitroprusside (SNP) (Kemika, Zagreb, Croatia) were added to isolated mesenteric arteries of normotensive and SH rats which were previously contracted by phenylephrine (10^{-6} M). The same procedure was repeated in the presence of different concentrations (0.1, 1, 10, 100 μg/ml) of MnSOD (isolated from *E. coli* by us) in the medium.

MnSOD was added to the medium 5 min before adding SNP. Acetylcholine, SNP, phenylephrine and all other chemicals were used without additional purification.

SOD isolation and enzyme assay

MnSOD from *E. coli* was isolated according to Keele [11]. The activity of SOD was assayed using the adrenaline method [15].

Statistical analysis

All data are expressed as the mean ± SEM and differences between groups were considered statistically significant when $p < 0.05$. One-way analysis of variance (ANOVA) followed by Dunnett's test and Newman-Keuls' test were used for comparisons between different concentrations and multiple dose-response curves, respectively.

Results

The effect of SNP on the relaxation of isolated mesenteric arteries from normotensive and SH rats with and without endothelium and the influence of MnSOD on the relaxing effect of SNP was examined in all (4) experimental groups.

SNP-mediated relaxation was stronger in normotensive compared to SH rats. SNP (10^{-5} M) caused 190% relaxation in the presence of the endothelium and 325% relaxation without the endothelium ($p < 0.05$)

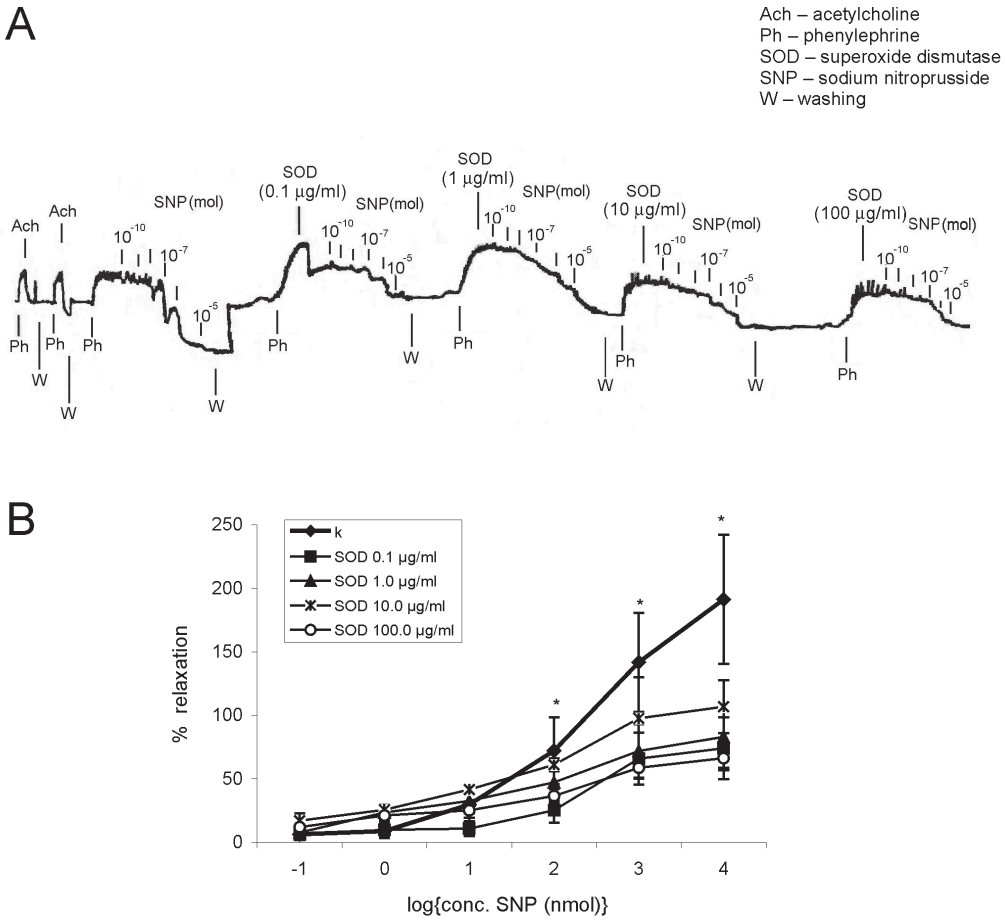

Fig . 1. (A) A representative recording of SNP-mediated relaxation of the mesenteric artery with endothelium (E+) isolated from normotensive rats, pre-contracted by phenylephrine (Ph), in the presence of different concentrations of MnSOD (*E. coli*) in the medium. **(B)** Dose-response curves for relaxation of rat mesenteric arteries of normotensive rat (E+) induced by increasing concentrations of SNP (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) in the presence of different concentrations (0.1, 1, 10, 100 µg /ml) of MnSOD (*E. coli*) in the medium. Data are the mean ± SEM of 6 experiments. * $p < 0.05$ compared with control (k)

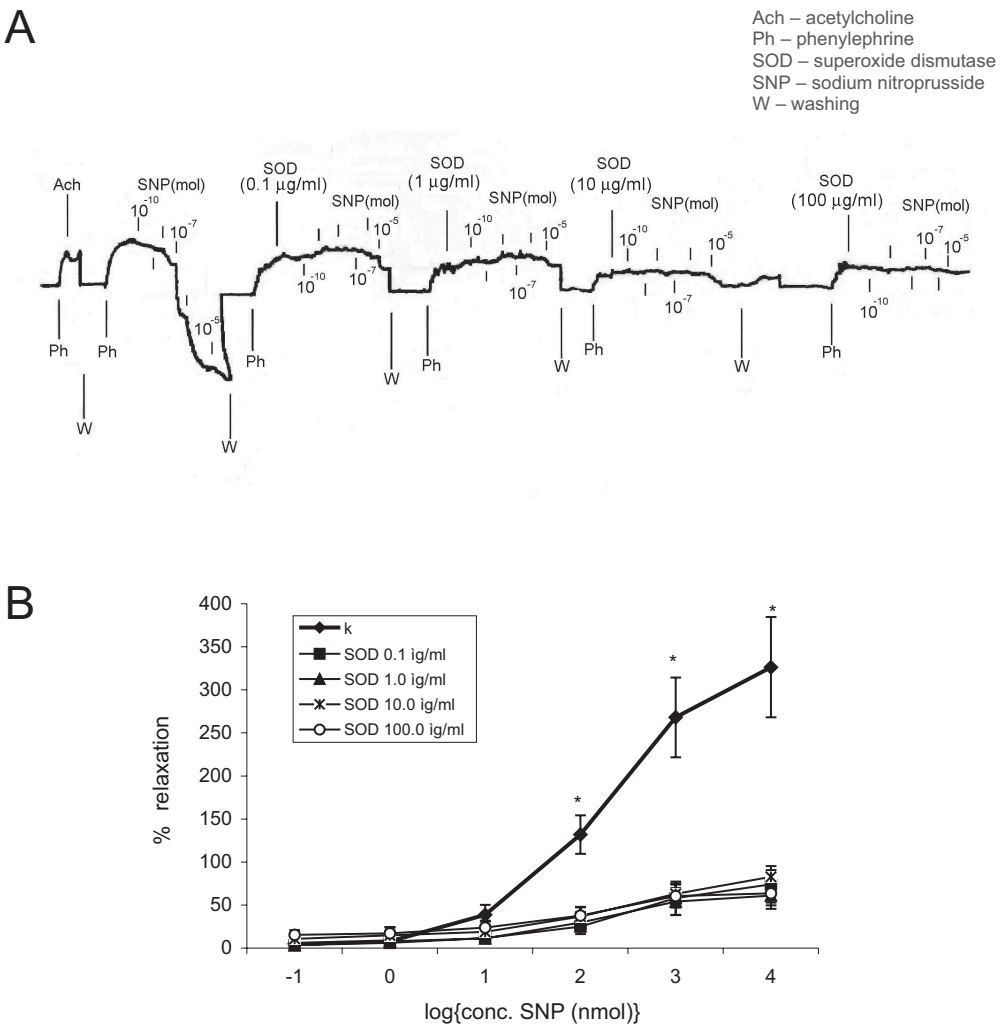

Fig. 2. (A) A representative recording of SNP-mediated relaxation of the mesenteric artery without endothelium (E-) isolated from normotensive rats, pre-contracted by phenylephrine (Ph), in the presence of different concentrations of MnSOD (*E. coli*) in the medium. **(B)** Dose-response curves for relaxation of rat mesenteric arteries of normotensive rat (E-) induced by increasing concentrations of SNP (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) in the presence of different concentrations (0.1, 1, 10, 100 µg /ml) of MnSOD (*E. coli*) in the medium. Data are the mean ± SEM of 6 experiments. * $p < 0.05$ compared with control (k)

in normotensive rats, and 35% relaxation in the presence of the endothelium and 135% relaxation without it ($p < 0.05$) in SH rats. The percentage of relaxation was greater than 100% because SNP is a strong vasodilator and causes relaxation that is below the basal tonus that is considered to be 100% relaxation. The relaxing effect of SNP on the isolated mesenteric artery from normotensive rats was greater without endothelium (Fig. 1A, 2A), and was greater than in isolated mesenteric artery from SH rats (Fig. 3A, 4A).

MnSOD exhibited a significant inhibitory effect on SNP-induced relaxation both in normotensive and SH rats except for mesenteric arteries from SH rats with endothelium where MnSOD potentiated SNP-induced relaxation (Fig. 3B).

Figures 3B and 4B indicate that the inhibitory effect of MnSOD on relaxation induced by SNP was less apparent in SH rats compared to normotensive rats. For example, the relaxing effect of SNP in normotensive rats was decreased 120% and 255% by the highest concentration (100 µg/ml) of MnSOD in comparison with control ($n = 6$, $p < 0.05$) in the presence and absence of endothelium, respectively. In SH rats, this inhibitory effect was 0–55% ($n = 6$, $p < 0.05$). MnSOD exhibited its strongest antagonistic effect in isolated mesenteric arteries without endothelium iso-

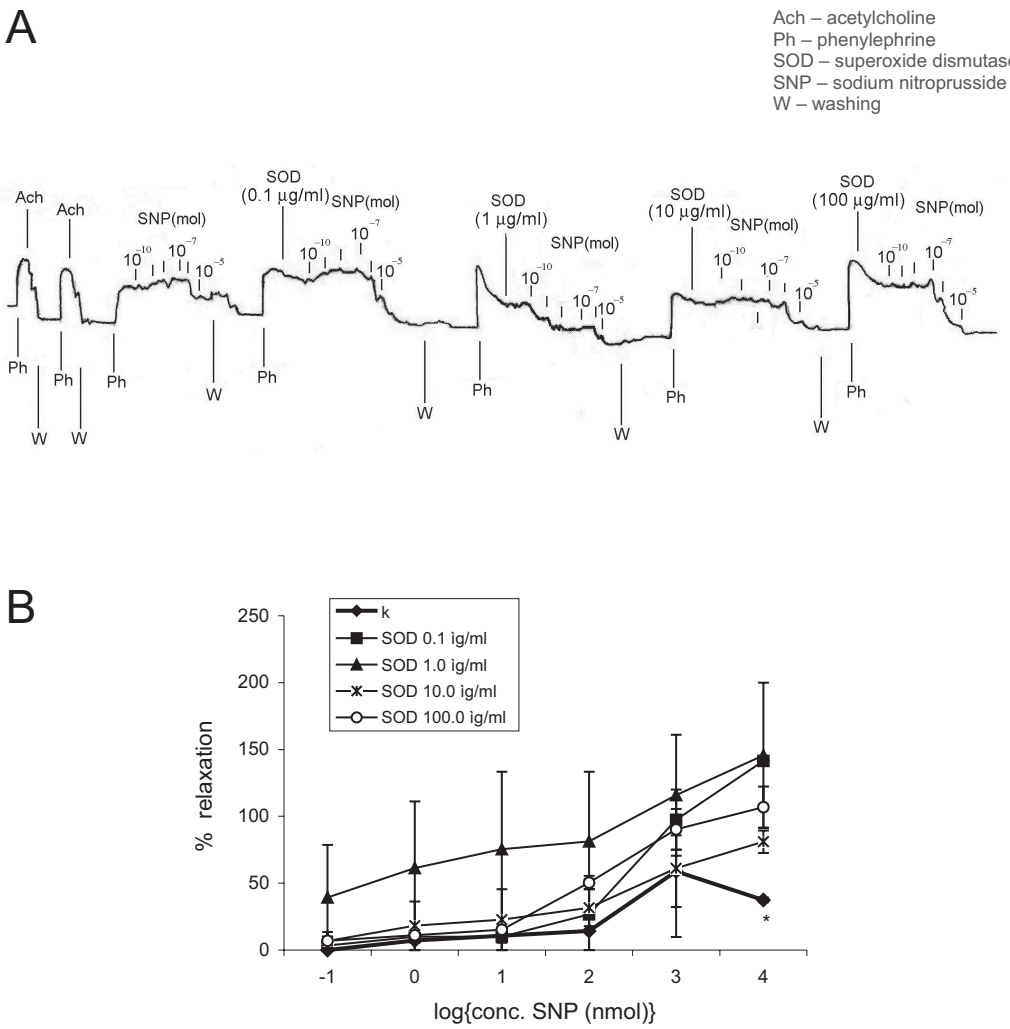

Fig. 3. (A) A representative recording of SNP-mediated relaxation of the mesenteric artery with endothelium (E+) isolated from SH rats, precontracted by phenylephrine (Ph), in the presence of different concentrations of MnSOD (*E. coli*) in the medium. **(B)** Dose-response curves for relaxation of rat mesenteric arteries of SH rat (E+) induced by increasing concentrations of SNP (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) in the presence of different concentrations (0.1, 1, 10, 100 µg /ml) of MnSOD (*E. coli*) in the medium. Data are the mean ± SEM of 6 experiments. * $p < 0.05$ compared with control (k)

lated from normotensive rats (Fig. 1A). No such inhibitory effect of MnSOD was found in isolated mesenteric arteries with endothelium isolated from SH rats (Fig. 3A).

Discussion

Previous studies [21] have already established that mesenteric arterial rings isolated from hypertensive rats show *in vitro* attenuated relaxation in response to SNP compared to rings from normotensive rats. However, those results suggest that the total functional capacity of vascular smooth muscle to relax in response to nitrovasodilators is not changed with aging or hypertension. In some previously published experiments, we demonstrated that isolated arteries responded differently to $NO^{\bullet}$ and its redox congeners [12]. In our current work, we have tested the response of mesenteric arteries isolated from normotensive rats to SNP in the presence and absence of exogenously added MnSOD. By conducting parallel experiments using the mesenteric artery isolated from SH rats, we were able to perform full comparison.

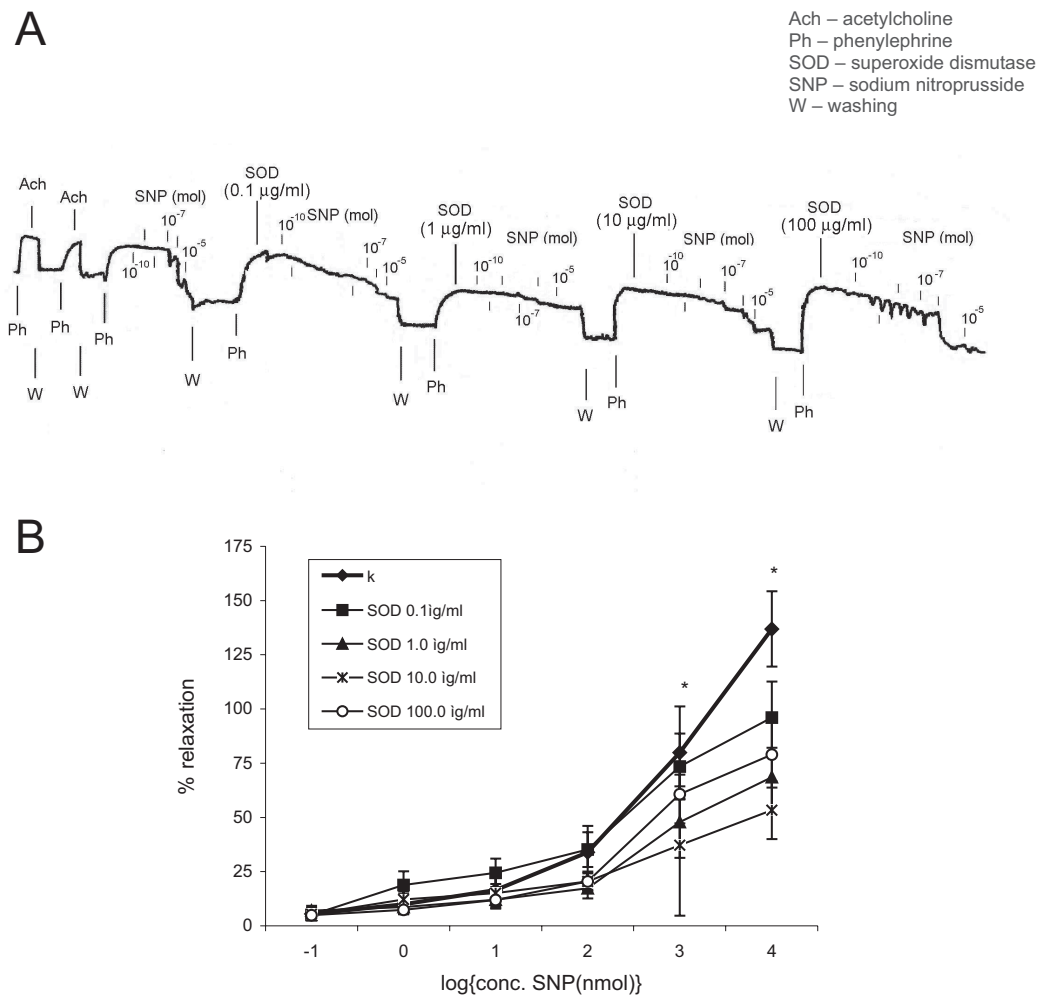

Fig. 4. (A) A representative recording of SNP-mediated relaxation of the mesenteric artery without endothelium (E-) isolated from SH rats, precontracted by phenylephrine (Ph), in the presence of different concentrations of MnSOD (*E. coli*) in the medium. **(B)** Dose-response curves for relaxation of rat mesenteric arteries of SH rat (E-) induced by increasing concentrations of SNP (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) in the presence of different concentrations (0.1, 1, 10, 100 µg/ ml) of MnSOD (*E. coli*) in the medium. Data are the mean ± SEM of 6 experiments. * $p < 0.05$ compared with control (k)

Herein we demonstrate a greater SNP-mediated relaxation of mesenteric arteries isolated from normotensive rats compared with that seen in mesenteric arteries isolated from SH rats. Similar results were obtained in experiments that involved SNP-mediated relaxation of isolated mesenteric arteries without endothelium. This indicated that besides the difference in the function of the endothelium, concerning basal $NO^{\bullet}$ production in normotensive and SH rats, a hypertension-induced difference existed in the smooth muscle with respect to $NO^{\bullet}$-mediated relaxation. The application of SODs in medicine is based on its enzymatic activity capable of eliminating $O_2^{\bullet-}$ and forming H_2O_2. One important effect of this enzyme is the protection against the breakdown of endothelium-derived vascular relaxation factor, $NO^{\bullet}$. The pharmacological effects of various SODs have been examined [1] and the experimental data indicate that, apart from their SOD activity, SODs have some direct pharmacological effects. Some tested doses of liposomal CuZnSOD caused a decrease (10–20 mmHg) in blood pressure. Higher concentrations of liposomal CuZnSOD caused a dose-dependent relaxation of the phenylephrine-contracted isolated inferior mesenteric artery. Free CuZnSOD has been shown to cause a triphasic effect on blood pressure which may be explained by a mechanism that SOD supports a reversible reduction of HNO to $NO^{\bullet}$ in addition to $O_2^{\bullet-}$ scavenging. Further-

more, MnSOD was shown to directly interact with smooth muscle and through non-specific reaction with calcium channels to cause a mild relaxing effect [22]. Although all SODs are equal with respect to $O_2^{\bullet-}$ dismutation enzymatic activity [10], their differences in reaction with NO [19], indicate their different biological activity. Some authors [5] have used SOD to study the participation of $O_2^{\bullet-}$ in the vasodilator response induced by SNP without specifying the exact nature of the SOD. One must realize that while testing the effect of SOD on isolated smooth muscle one must take into account SOD's different reactivity towards $NO^{\bullet}$.

For this reason, we expected that in our experiments using isolated mesenteric arteries isolated from normotensive and SH rats, MnSOD would exaggerate SNP-mediated relaxation. However, our results indicated a significant inhibitory effect of MnSOD on SNP-mediated relaxation, except for mesenteric arteries containing endothelium isolated from SH rat, that is in accordance with experiments performed by Grunfeld et al. [7]. The ability of SOD to decrease $O_2^{\bullet-}$ and increase $NO^{\bullet}$ release was greater in SH rats compared to normotensive rats.

Another possible explanation of our results may be that MnSOD converted $NO^{\bullet}$ into HNO/NO^- and reduced cGMP-dependent relaxation. Therefore, cGMP-independent HNO/NO^--mediated relaxation may be operative *in vivo*. It seems that this predominates in SH rats, compared with normotensive rats indicating again that vascular dysfunction in SH rats is not solely endothelial-derived but involves other components such as vascular smooth muscle. The generation of NO^+ and NO^- ions by $NO^{\bullet}$-treated MnSOD, which produces both enzyme modifications and inactivation, has been demonstrated [6, 19]. NO^- is a redox-sensitive positive inotrope with selective vasodilatory action [20]. Furthermore, the differential chemical behavior of $NO^{\bullet}$ and NO^- toward heme proteins offers the unique control mechanism for the biological action of $NO^{\bullet}$ [23]. On the other hand, the diverse physiological responses observed following exposure of cells and/or isolated organs to $NO^{\bullet}$ and HNO/NO^- donors suggest an intriguing and novel condition-dependent dimension to signal transduction and cellular regulation [18]. HNO/NO^- induces CGRP release leading to the activation of CGRP receptors and subsequent cAMP-dependent smooth muscle relaxation [2, 3, 18].

In conclusion, our results demonstrated greater SNP-mediated relaxation of mesenteric arteries from normotensive rats compared to SH rats. Similar results were obtained using isolated mesenteric arteries without endothelium, indicating that besides the difference in the function of endothelium, concerning basal $NO^{\bullet}$ production in normotensive and SH rats, there is a distinction in the smooth muscle that is induced by hypertension with respect to $NO^{\bullet}$ relaxation. The presence of MnSOD in the Krebs-Ringer bicarbonate solution abrogated SNP-mediated relaxation in all the examined artery preparations except for the mesenteric artery isolated from SH rats with endothelium, which shows that mechanisms of artery relaxation are different due to an endothelial and smooth muscle changes in SH, as compared to normotensive rats. Our results clearly show that MnSOD, by modifying the chemical versatility of $NO^{\bullet}$ into redox active forms, (NO^+ and HNO/NO^-), results in differential relaxing effects in the arteries of normotensive and SH rats (with or without the endothelium), suggesting the possible role of HNO/NO^--induced relaxation in SH rats.

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