

J. Serb. Chem. Soc. 69 (11) 973–980 (2004)
JSCS – 3225

UDC 714–31+546.17–31+546.33:616.61–005
Original scientific paper

Effect of MnSOD (*E. coli*) on the relaxation caused by sodium nitroprusside on isolated rat renal artery*

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(Received 20 May 2004)

Abstract: In this study the molecular foundation of nitric oxide induced relaxation of arteries, with or without endothelium, of normotensive and spontaneously hypertensive rats was re-examined. With this purpose in mind, the effects of the nitric oxide donor sodium nitroprusside (NaNP), with and without manganese containing superoxide dismutase (MnSOD E.C. 1.15.1.1.), on rat renal artery relaxation was studied. The results show that the relaxation effect of NaNP is two times higher in normotensive, compared to spontaneously hypertensive rats. Similar differences exist in the relaxation effects of NaNP on isolated renal arteries without endothelium, indicating that besides the difference in the function of an endothelium, concerning basal NO production in normotensive and hypertensive rats, there is a difference with respect to NO relaxation in the smooth muscle that is induced by hypertension. MnSOD decreased the relaxation effect of NaNP in all the examined renal arteries, more in normotensive than in hypertensive ones regardless of the presence of an endothelium. These results show that MnSOD, by modifying the chemical versatility of NO into redox active forms - nitrosonium (NO⁺) and nitroxyl (NO⁻), produces different relaxation effects in normotensive and hypertensive arteries of rats, with or without an endothelium, potentiating the role of nitroxyl induced relaxation in spontaneously hypertensive rats. The results prove the need for the synthesis of complex NO donors, as the mechanisms of artery relaxation are different due to an endothel and smooth muscle changes in hypertensive, as compared to normotensive rats.

Keywords: sodium nitroprusside, manganese superoxide dismutase, renal artery, spontaneously hypertensive rats.

INTRODUCTION

Endothelial dysfunction reduces endogenous bioactivity and identifies NO signaling as a key target for therapeutic intervention to preserve tissue integrity and minimize irreversible damage associate with hypertension and ischemic cardio-

* Dedicated to Professor Živorad Čeković on the occasion of his 70th birthday.

Serbian Chemical Society active members.

vascular diseases.¹ Diverse physiological and pathophysiological phenomena caused by nitric oxide (NO) are mediated through guanosine 3',5'-cyclic monophosphate (cGMP) dependent pathways or through cGMP-independent pathways,² NO signals, initiated by activation of guanylyl cyclase, are amplified by accumulated cGMP-propagated within cells by downstream effectors, primarily, GMP-dependent protein kinases, cyclic nucleotide-gated channels, and phosphodiesterases (PDEs). cGMP-dependent protein kinases, serine-threonine kinases, the activation of which is controlled by the binding of cGMP to allosteric regulatory domains, mediate smooth muscle relaxation by antagonizing the calcium required for contraction, reflecting inhibition of extracellular calcium influx and intracellular calcium release, and desensitization of the contractile apparatus to calcium. Other pathways are mediated through the formation of nitrosothiol adducts (RS-NOs). The proposed pathways for S-nitrosothiol (RSNOs) formation include the reaction of thiols with dinitrosyl-iron or nitrosylheme complexes or direct reaction of thiols with NO in presence of an electron acceptor, such as NAD⁺.³

It was reported that the oxidative transformation of the free hydroxyl group of isosorbide-5-mononitrate (IS-5-MN) leads to the corresponding keto-nitrate of isosorbide, which was then converted to the isosorbide-5-nitrate-2-ketoxime.⁴ The synthesized oximenitrate (10^{-8} – 10^{-6} mol/L) also induces a concentration-dependent relaxation in a precontracted preparation of the superior mesenteric artery contracted by phenylephrine, but this inhibitory effect was statistically more intensive than that caused by IS-5-MN. It appears that the newly synthesized oxime nitrate is more effective as a vasodilating agent and it has been suggested that the new nitro compound could be of interest in the treatment of angina caused by coronary artery spasms.⁵

In this study the effect of NO donors on relaxation of artery with respect to the chemical nature of NO donated was examined. One of these, sodium nitroprusside (NaNP) is an NO/NO⁺ donor which is used clinically to treat hypertensive emergencies.⁶ The mechanism of NO release from NaNP is still unknown. However, it is believed that in biological systems both enzymatic and nonenzymatic single-electron reductions may be involved. Another goal of this study was to investigate how the generation of nitrosonium (NO⁺) and nitroxyl (NO⁻) ions, from NO liberated from NaNP in the presence of MnSOD,³ influence the effect of NaNP.

EXPERIMENTAL

Experiments were performed on renal arteries isolated from male Wistar rats and spontaneously hypertensive (SH) rats, (250–300 g).

Adhering perivascular tissue was removed carefully from the arteries which were then cut into 3–5 mm rings, incubated 30 min in water bath in Krebs-Ringer bicarbonate solution at 36 °C, and then gassed with 95 % O₂ and 5 % CO₂. Each ring was gradually stretched to the optimal tension (2 g) and an isometric transducer registered the mechanical contractions.

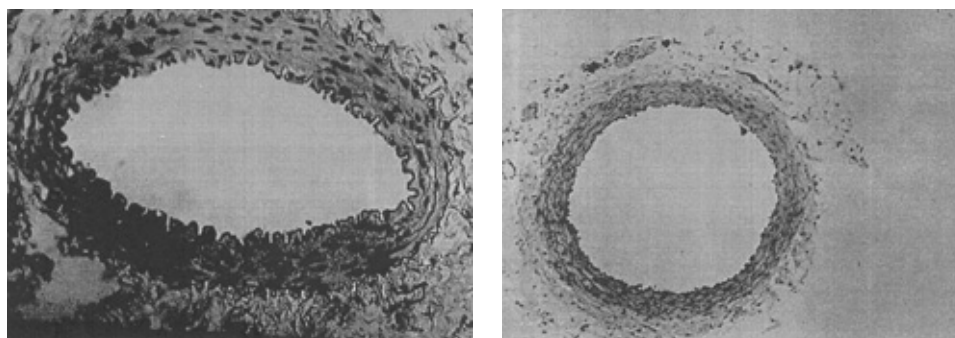
Contractions of the isolated blood vessels were induced by phenylephrine (10^{-6} mol) and the functional integrity of the endothelium was confirmed by acetylcholine (10^{-5} mol) and histopathologic examination. Preparations were analyzed using a microscope to distinguish differences between blood vessels of normotensive and hypertensive rats.

Blood vessels isolated from both normotensive and hypertensive rats were analyzed in three different situations: just isolated blood vessels; blood vessel contracted by phenylephrine; blood vessel relaxed (or not if the endothelium was not preserved) by acetylcholine.

TABLE I. Percent of relaxation of the rat renal arteries with and without an endothelium in the presence of increasing concentrations of NaNP without and with 10 µg/ml MnSOD. The precontractions were induced by phenylephrine (10^{-6} M)

Renal artery	Endothel	NaNP	NaNP + MnSOD
Normotensive	E+	$y = 37.985x - 59.687$	$y = 2.1057x + 0.8232$
	E-	$y = 49.735x - 96.247$	$y = 5.3568x - 6.0781$
Hypertensive	E+	$y = 18.257x - 15.678$	$y = 15.167x - 26.111$
	E-	$y = 14.908x - 21.062$	$y = 8.3306x - 12.052$

Histopathological analysis (Figs. 1a and b) shows the endothelium was better preserved in normotensive than in hypertensive rats. The percentage relaxation caused by acetylcholine depended on the degree of endothelial preservation. In hypertensive rats the endothelium is spontaneously damaged due to high blood pressure, so the relaxing effect of acetylcholine is much lower than in normotensive rats.



a) Renal artery of a normotensive rat

b) Renal artery of a hypertensive rat

Fig. 1. Renal artery of a) a normotensive, b) a spontaneously hypertensive rat.

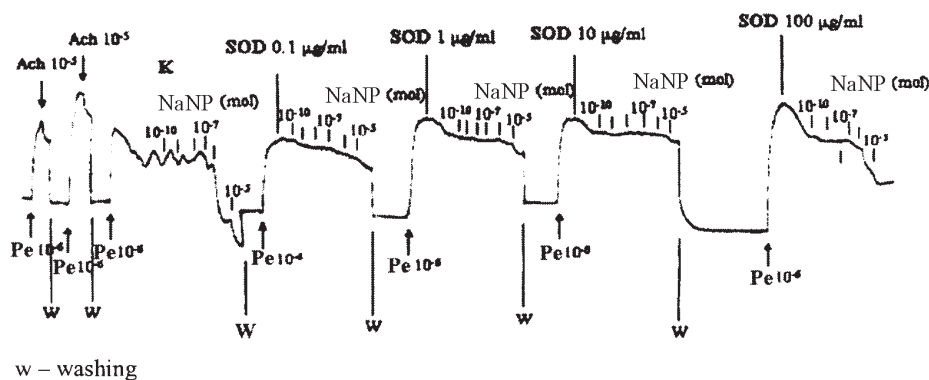
Superoxide dismutase assay and isolation and purification

Sodium nitroprusside, acetylcholine, phenylephrine and all other chemicals were of reagent grade and were used without additional purification. MnSOD (*Escherichia coli*) was isolated according to Keele.⁷

The activity of SOD was assayed by the adrenaline method.⁸

RESULTS AND DISCUSSION

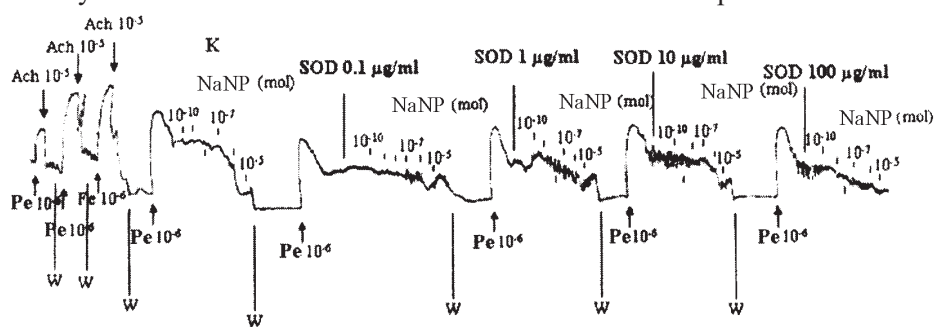
The application of superoxide dismutases in medicine is based on their enzyme activity during which the superoxide anion radical ($O_2^{\cdot-}$) is removed. One important effect is protection against breakdown of the endothelium-derived vascular relaxation factor – nitric oxide (NO).⁹ The pharmacological effects of various SODs have been examined¹⁰ and experimental data indicate that, apart from superoxide dismutational activity, SODs have certain direct pharmacological ef-



w - washing

Fig. 3. A representative trace of the responses of an isolated renal artery from a normotensive rat without an endothelium (E-). The renal artery was precontracted with phenylephrine - Pe (10^{-6} M) then relaxed with acetylcholine - Ach (10^{-5} M) and after washing another contraction was induced with Pe (10^{-6} M) and treated with increasing concentrations of sodium nitroprusside - NaNP (10^{-10} M, 10^{-9} M, 10^{-8} M, 10^{-6} M and 10^{-5} M). After washing the same protocols were repeated in the presence of different concentrations of MnSOD (0.1 μ g/ml; 1 μ g/ml; 10 μ g/ml and 100 μ g/ml) in the medium.

which helps to explain some of the opposing effects of SODs observed in NOS-mediated events. Nitroxyl anions liberate the 37 amino acid peptide, a calcitonine gene related peptide (CGRP), which 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 *in vivo*. In patients suffering from heart failure (HF), CGRP has been shown to reduce pulmonary and systemic pressure and to increase cardiac performance without producing tachycardia.¹⁶ A possible explanation of our results may be that MnSOD eliminates NO and reduces cGMP dependent relaxation.



w - washing

Fig. 4. A representative trace of the responses of an isolated renal artery from a hypertensive rat with an endothelium (E+). The renal artery was precontracted with phenylephrine - Pe (10^{-6} M) then relaxed with acetylcholine - Ach (10^{-5} M) and after washing another contraction induced with Pe (10^{-6} M) and treated with increasing concentrations of sodium nitroprusside - NaNP (10^{-10} M, 10^{-9} M, 10^{-8} M, 10^{-6} M and 10^{-5} M). After washing the same protocols were repeated in the presence of different concentrations of MnSOD (0.1 μ g/ml; 1 μ g/ml; 10 μ g/ml and 100 μ g/ml) in the medium.

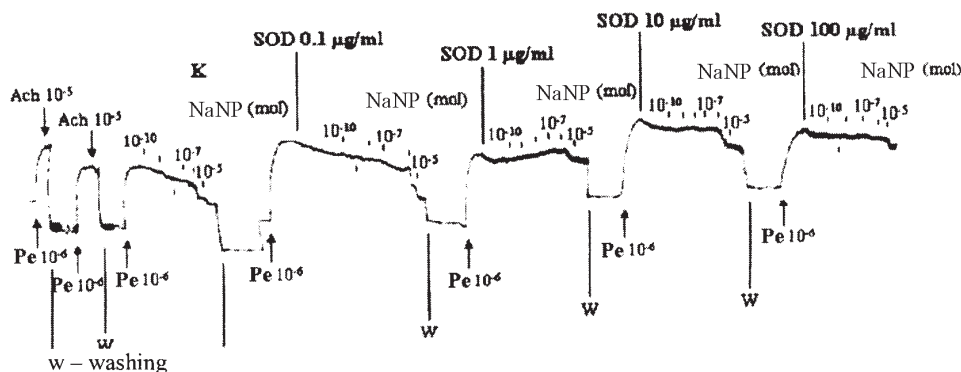


Fig. 5. A representative trace of the responses of an isolated renal artery from a hypertensive rat without endothelium (E-). The renal artery was precontracted with phenylephrine – Pe (10^{-6} M) and then relaxed with acetylcholine – Ach (10^{-5} M) and after washing another contraction was induced with Pe (10^{-6} M) and treated with increasing concentrations of sodium nitroprusside – NaNP (10^{-10} M, 10^{-9} M, 10^{-8} M, 10^{-6} M and 10^{-5} M). After washing the same protocols were repeated in the presence of different concentrations of MnSOD (0.1 $\mu\text{g/ml}$; 1 $\mu\text{g/ml}$; 10 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$) in the medium.

Another mechanism, none cGMP dependent, is that nitroxyl anion mediated relaxation may be operative *in vivo*. It seems that this is more expressed in spontaneously hypertensive than in normotensive rats, indicating that vascular dysfunction in spontaneously hypertensive rats is not solely endothelial – derived but involve other components, such as the vascular smooth muscle.

CONCLUSION

The presented results show a two times higher relaxation effect of NaNP in normotensive, compared to spontaneously hypertensive rats. Similar differences exist in the relaxation effects of NaNP on isolated renal arteries without an endothelium, indicating that besides the difference in the function of endothelium, concerning basal NO production in normotensive and hypertensive rats, there is a difference in the smooth muscle that is induced by hypertension with respect to NO relaxation. The presence of MnSOD decreases the relaxation effect of NaNP in all the examined renal arteries, more in normotensive than in hypertensive ones, regardless of the presence of an endothelium. These results show that MnSOD, by modifying chemical versatility of NO into redox active forms – nitrosonium (NO^+) and nitroxyl (NO^-), produces different relaxation effects in the arteries of normotensive and hypertensive rats with or without an endothelium, potentiating the possible role of nitroxyl induced relaxation in spontaneously hypertensive rats.

Acknowledgements: This work is part of the research activities of the projects 1669, 1740 and 0295. The authors would like to thank the Ministry of Science, Tehnology and Development of the Republic of Serbia for financial support of the projects.

ИЗВОД

ЕФЕКАТ MnSOD (*E. coli*) НА РЕЛАКСАЦИЈУ ИЗОЛОВАНЕ РЕНАЛНЕ АРТЕРИЈЕ ПАЦОВА ИЗАЗВАНУ НАТРИЈУМ-НИТРО-ПРУСИДОМСЛОБОДАН МИЛОВАНОВИЋ¹, ЗОРАНА ОРЕШЧАНИН², СНЕЖАНА СПАСИЋ³, СРЉАН МИЛЕТИЋ³, МИЛИЦА ПРОСТРАН⁴ И МИХАЈЛО Б. СПАСИЋ⁵

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У овом раду смо покушали да детаљније испитамо молекулску основу азотоксид индуковане релаксације артерија, са и без ендотела, нормотензивних и спонтано хипертензивних пацова. У ту сврху испитиван је ефекат азот-оксид (NO) донора натријум-нитроприсида (NaNP), без и у присуству супероксид-дисмутазе која садржи манган (MnSOD, ЕС 1.15.1.1.) на релаксацију реналне артерије. Резултати показују двоструко већи релаксантни ефекат NaNP код нормотензивних, у односу на спонтано хипертензивне пацове. Слична разлика постоји и у релаксантном ефекту NaNP на изоловане реналне артерије нормотензивног и хипертензивног пацова када је одстрањен ендотел што указује да, поред разлике у функцији ендотела у односу на базалну NO продукцију код нормотензивних и спонтано хипертензивних пацова, постоје и промене у глатким мишићима индуковане хипертензијом, у односу на NO релаксацију. MnSOD код свих група смањује релаксантни ефекат SNP и то више код нормотензивних него код хипертензивних, независно од присуства ендотела. Ови резултати показују да MnSOD мењајући хемијску природу из NaNP ослобођеног NO у редокс активне форме – нитрозо-нијум (NO⁺) и нитроксил (NO⁻) – остварује различит релаксантни ефекат код нормотензивних и код хипертензивних пацова са и без ендотела потенцирајући значај нитроксилем индуковане релаксације код спонтано-хипертензивних пацова. Добијени резултати потврђују потребу синтезе NO донора који дају различите редокс-активне форме NO будући да се примарни механизми релаксације разликују у зависности од карактеристика ендотела и глатких мишића, код хипертензије у односу на нормотензивно стање.

(Примљено 20. маја 2004)

REFERENCES

1. L. Gong, G. M. Pitari, S. Schulz, S. A. Waldman, *Curr. Opin. Hematol* **11** (2004) 1
2. B. P. Booth, M. A. Tabrizi-Fard, H. L. Fung, *Bioch. Pharmac.* **59** (2000) 1603
3. V. Niketić, S. Stojanović, A. Nikolić, M. Spasić, A. M. Michelson, *Free Rad. Biol. & Med.* **27** (1999) 992
4. Z. T. Tokić-Vujošević, Ž. Čeković, *Synthesis* **13** (2001) 2028
5. R. M. Stepanović Petrović, Z. Tokić Vujošević, S. Milovanović, Ž. Čeković, D. Bokonjić, S. Dobrić, *Pharmazie* **57** (2002) 507
6. Y. C. Hou, A. Jancyk, P. G. Wang, *Current Pharmaceutical Design* **5** (1999) 417
7. B. V. Keele, J. M. McCord, I. Fridovich, *J. Biol. Chem.* **245** (1970) 6176
8. H. P. Misra, I. Fridovich, *J. Biol. Chem.* **247** (1972) 3170
9. V. R. Muzykantov, *J. Controlled Release* **71** (2001) 1
10. M. B. Spasić, S. Milovanović, M. Simović, V. M. Petrović, A. M. Michelson, The effects of superoxide dismutase on isolated smooth muscle of the rat in *Anticarcinogenesis and Radiation Protection* **2**, O. F. Nygaard, A. C. Upton, Plenum Press, New York, 1991, p. 345
11. S. Milovanović, M. Simović, M. Cvetković, A. Milovanović, M. Spasić, *Jugoslavica physiol. pharmacologica acta* **24** (1988) 57

12. M. E. Murphy, H. Sies, *Proc. Natl. Acad. Sci. USA* **88** (1991) 10860
13. N. Paolucci, W. F. Saavedra, K. M. Miranda, C. Martignani, T. Isoda, J. M. Hare, M. G. Espey, J. M. Fukuto, M. Feelisch, D. A. Wink, D. A. Kass, *PNAS* **18** (2001) 10463
14. M. G. Espey, K. M. Miranda, D. D. Thomas, D. A. Wink, *Free Rad. Biol. & Med.* **33** (2002) 827
15. S. P. Didion, D. D. Heistad, F. M. Faraci, *Stroke* **32** (2001) 761
16. K. M. Miranda, R. W. Nims, D. D. Thomas, M. G. Espey, D. Citrin, M. D. Bartberger, N. Paolucci, J. M. Fukuto, M. Feelisch, D. A. Wink, *J. Inorg. Biochem.* **93** (2003) 52.