UDC 577
Original scientific paper

METABOLISM OF SODIUM NITROPRUSSIDE (SNP) IN RAT RED BLOOD CELLS

Snežana D. MALETIĆ[1], Branka I. OGNJANOVIĆ[1], Andraš Š. Štajn[1], Radoslav V. ŽIKIĆ[2], Zorica S. SAIČIĆ[2] and Mihailo B. SPASIĆ[2]

[1]Institute of Biology and Ecology, Faculty of Sciences, University of Kragujevac, Kragujevac,
[2]Institute for Biological Research "Siniša Stanković", Department of Physiology, Belgrade, Serbia and Montenegro

Maletić D. Snežana, Branka I. Ognjanović, Andraš Š. Štajn, Radoslav V. Žikić, Zorica S. Saičić and Mihailo B. Spasić (2004): *Metabolism of sodium nitroprusside (SNP) in rat red blood cells.* - Iugoslav. Physiol. Parmacol. Acta, Vol. 40, No. 1-3, 37-42, Belgrade.

Sodium nitroprusside (SNP) is an established drug, clinically used in the treatment of hypertensive emergencies. The aim of this study is further elucidation of the molecular mechanism of the SNP action, particularly its metabolism in rat erythrocytes and reticulocytes. Rats erythrocyte and reticulocyte-rich suspensions were aerobicaly incubated without (control) or in the presence of SNP (0.1, 0.25, 0.5, 1.0 i 1.5 mM). The concentrations of reactive nitrogen species (RNS) and reactive oxygen species (ROS) were determined after incubation. In rat erythrocytes, SNP did not alter nitrite level (NO^+ ion indicator), while significantly increased concentrations of hydroxylamine (NO^- ion indicator), S-nitrosothiols (SNO) and 3-nitrotyrosine (peroxynitrite indicator). Concentration of superoxide anion ($O2^{\cdot-}$) decreased in the

Corresponding author: Ass. Snežana D. Maletić, MSc., Molecular Biologist and Physiologist, Institute of Biology and Ecology, Faculty of Sciences, Radoje Domanović 12, 34000 Kragujevac, POB. 60, Serbia and Montenegro; Tel: ++ 381 34 336 223 (203); Fax: ++381 34 335 040; E-mail: maletic@knez.uis.kg.ac.yu

presence of low doses of SNP only, while level of hydrogen peroxide (H_2O_2) increased in dose-dependent manner in rat erythrocytes. On the other hand, SNP significantly increased nitrite, hydroxylamine and 3-nitrotyrosine concentrations in rat reticulocytes. In addition, low doses of SNP induced decrease of $O_2^{\cdot-}$ level. Concentration of H_2O_2 did not alter in rat reticulocytes. On the basis of these data, we can conclude: SNP spontaneously liberated nitric oxide as NO^- ion in rat erythrocytes and reticulocytes. In addition, applied experimental doses of SNP induced strong nitrosative and oxidative stress in these cells.

Key words: erythrocytes, nitroxyl anion, reticulocytes, sodium nitroprusside.

INTRODUCTION

Corresponding to their intermediate position in the differentiation program, the reticulocytes do not possess full range of metabolic pathways of proliferating cells, but they are still equipped with a set of metabolic pathways (corresponding to presence of mitochondria and ribosomes), most of which are lost during their transition to the mature erythrocytes (RAPOPORT, 1986). Reactive oxygen species (ROS) and their derivatives are present in living tissues at low but measurable concentrations, which are determined by the balance between the rates of radical production and their corresponding rates of clearance (DROGE, 2002). In conditions of disturbed prooxidant-antioxidant balance in favour of the prooxidants, leading to potential damage of cells, the oxidative stress occurs (SIES, 1991). Nitric oxide (NO) is a small hydrophobic molecule with chemical properties which make it uniquely suitable for both intra- and intercellular messenger. In the reactions with $O_2^{\cdot-}/O_2$, NO generated reactive nitrogen species (RNS) which affected almost every molecule in cells (WINK and MITCHELL, 1998). Diverse and important physiological roles of NO implicate that exogenous donation of NO may be useful in the treatment of some disease states. Transition metal NO complex, sodium nitroprusside (SNP) spontaneously releases NO, mainly as nitrosonium ion - NO^+ (HOU *et al.*, 1999).

The aim of this study is further elucidation of the molecular mechanism of the SNP action, particularly its metabolism in rat erythrocytes and reticulocytes.

MATERIAL AND METHODS

Erythrocyte and reticulocyte-rich red blood cell suspensions of rats (Wistar albino rats of 250-350 g body mass) were used in this study. Reticulocytosis was induced by phenylhydrazine hydrochloride treatment (35 mg/kg body mass during three days) (KOSTIĆ *et al.*, 1990). After 7-8 days, rats were anaesthetized by ether, and blood was taken by exanguination. Reticulocytes amounted to 86.57±1.28 %. Three times washed red blood cells were resuspended in incubation buffer containing: 50 mM Hepes, 100 mM NaCl, 1 mM $MgCl_2$, 1 mM NaH_2PO_4, 5

mM glucose and 2 mM $CaCl_2$, pH 7.4 at 37°C (KOSTIĆ *et al.*, 1990). Cell suspensions (final hematocrit value about 0.20) were aerobically incubated for 2 hours, without (control), or in the presence of different concentrations of SNP: 0.1, 0.25, 0.5, 1.0 and 1.5 mM. The SNP was added at the beginning of incubation (0 min). Samples extractions were carried out after incubation.

Concentrations of RNS and ROS were determined in L-Arg extracts: ½ vol 3 M perchloacetic acid and 2 vol 20 mM EDTA were added to 1 vol cell suspension. After extraction on ice (15 min) and centrifugation 4 min/15 000 rpm, extracts were neutralized by 2 M K_2CO_3. Spectrophotometric determination of nitrites (NO^+ indicator) (GREEN *et al.*, 1982), hydroxylamine (NO^- indicator) (ARNELLE and STAMLER, 1996), S-nitrosothiols (SNO) (GREEN *et al.*, 1982), 3-nitrotyrosine - (peroxynitrite indicator) (RIORDAN and VALLEE, 1972), superoxide anion ($O_2^{\cdot-}$) (AUCLAIR and VOISIN, 1985) and hydrogen peroxide (H_2O_2) concentrations (PICK and KEISARI, 1980) were performed in L-Arg extracts.

All values are expressed as mean ± SEM. Statistical evaluation was calculated by Student's t-test for paired observations. For all comparisons $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Results presented in Figure 1 showed that SNP did not alter nitrite level, while significantly ($p < 0.05$) increased concentrations of hydroxylamine, S-nitrosothiols and 3-nitrotyrosine in rat erythrocytes. Literature data (HOU *et al.*, 1999) showed that SNP as transition metal NO complex, spontaneously releases NO, mainly as NO^+ ion. However, according to results of our study, there is dominant generation of nitroxyl anion (NO^-) due to SNP metabolism in rat erythrocytes. These data have new implications, because NO^- ion has discrete chemistry versus the other RNS and accomplish strong cytotoxic effects (MIRANDA *et al.*, 2003).

Concentration of $O_2^{\cdot-}$ decreased ($p < 0.05$) in the presence of low doses of SNP only (Fig. 2). These data is probably consequence of peroxynitrite generation (Fig. 1). Namely, NO in the reaction with $O_2^{\cdot-}$, as well as NO^- ion in reaction with O_2 generated peroxynitrite, which damaged almost every molecule in cells (WINK and MITCHELL, 1998). Level of H_2O_2 increased in dose-dependent manner in the presence of SNP (Fig. 2), indicating oxidative stress (SIES, 1991) that occurs in rat erythrocytes.

In rat reticulocytes SNP significantly increased nitrite, hydroxylamine and 3-nitrotyrosine concentrations (Fig. 3). Apparently, SNP is metabolized to NO^- ion in reticulocytes, too. Generation of RNS, indicate SNP-induced nitrosative stress in these cells. In addition, low doses of SNP induced decrease of $O_2^{\cdot-}$ level, while high doses of SNP even increased $O_2^{\cdot-}$ concentration (Fig. 4). Concentration of H_2O_2 did not alter in rat reticulocytes (Fig. 4). These alterations of ROS is consequence of SNP-induced inhibition of Cu, Zn-containing superoxid dismutase and Mn-containing superoxid dismutase (MALETIĆ *et al.*, unpublished data).

Our previous study has been showed SNP-induced inhibition of the process of oxidative phosphorylation in rat reticulocyte mitochondria (MALETIĆ *et al.*, 2004) and stimulation of the process of glycolysis in rat reticulocytes (MALETIĆ *et al.*, 2004) and erythrocytes (MALETIĆ *et al.*, 2000). Data presented now (current study) indicate that SNP-induced alteration of energy production in rat red blood cells are mediated dominantly by NO^- ion, as well as RNS and H_2O_2 (Fig. 1, 2, 3, 4).

On the basis of obtained data, we can conclude: SNP spontaneously liberated nitric oxide as NO^- ion in rat erythrocytes and reticulocytes. In addition, applied experimental doses of SNP induced strong nitrosative and oxidative stress in these cells.

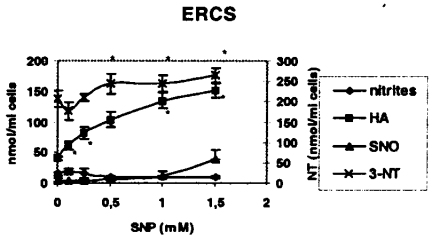

Fig. 1. - Alterations of nitrite, hydroxylamine, S-nitrosothiols and 3-nitrotyrosine concentrations in the presence of SNP in rat erythrocytes
Values represent mean ± SEM for 4 experiments
*p < 0.05, control (0 mM SNP) versus SNP (other concentrations)

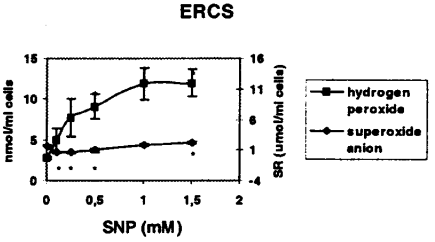

Fig. 2. - Alterations of superoxide anion radical and hydrogen peroxide concentrations in the presence of SNP in rat erythrocytes
Values represent mean ± SEM for 4 experiments
*p < 0.05, control (0 mM SNP) versus SNP (other concentrations)

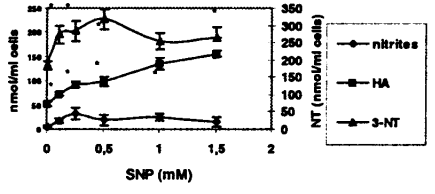

Fig. 3. - Alterations of nitrite, hydroxylamine and 3-nitrotyrosine concentrations in the presence of SNP in rat reticulocytes
Values represent mean ± SEM for 4 experiments
*p < 0.05, control (0 mM SNP) versus SNP (other concentrations)

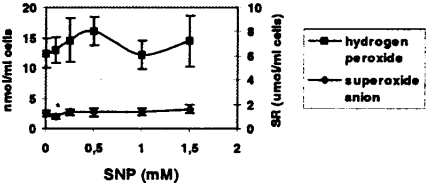

Fig. 4. - Alterations of superoxide anion radical and hydrogen peroxide concentrations in the presence of SNP in rat reticulocytes
Values represent mean ± SEM for 4 experiments
*p < 0.05, control (0 mM SNP) versus SNP (other concentrations)

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Recieved May 5, 2004
Accepted May 10, 2004

METABOLIZAM NATRIJUM NITROPRUSIDA (SNP) U CRVENIM KRVNIM ĆELIJAMA PACOVA

Snežana D. MALETIĆ[1], Branka I. OGNJANOVIĆ[1], Andraš Š. Štajn[1], Radoslav V. ŽIKIĆ[2], Zorica S. SAIČIĆ[2] i Mihailo B. SPASIĆ[2]

[1]Institut za biologiju i ekologiju, PMF, Univerzitet u Kragujevcu, Kragujevac,
[2]Institut za biološka istraživanja "Siniša Stanković",
Odeljenje fiziologije, Beograd, Srbija i Crna Gora

Natrijum nitroprusid (SNP) je lek koji se klinički koristi u tretmanu hipertenzija. Cilj ovog rada je da se objasne molekularni mehanizmi delovanja SNPa, sa posebnim akcentom na metabolizam ovog leka u eritrocitima i retikulocitima pacova. Suspenzije eritrocita i crvenih krvnih ćelija bogate retikulocitima su aerobno inkubirane bez (kontrola) ili u prisustvu različitih koncentracija SNPa (0.1, 0.25, 0.5, 1.0 i 1.5 mM). Koncentracije reaktivnih vrsta azota (RNS) i kiseonika (ROS) su određivane nakon inkubacije. U eritrocitima pacova, SNP ne menja koncentraciju nitrita (indikator NO^+ jona), dok značajno povećava koncentracije hidroksilamina (indicator NO^- jona), S-nitrozotiola (SNO) and 3-nitrotirozina (indikator peroksinitrita). Koncentracija superoksid anjon radikala ($O_2^{\cdot-}$) je smanjena samo u prisustvu niskih doza SNP, dok je nivo vodonik peroksida (H_2O_2) povećan na dozno-zavisan način u eritrocitima pacova. S druge strane, SNP značajno povećava koncentracije nitrita, hidroksilamina i 3-nitrotirozina u retikulocitima pacova. Niske doze SNPa indukuju smanjenje $O_2^{\cdot-}$ nivoa. Koncentracije H_2O_2 nisu promenjenje u retikulocitima pacova. Na osnovu iznetih podataka, možemo zaključiti: SNP spontano oslobađa azot monoksid kao NO^- jon u eritrocitima i retikulocitima pacova. Primenjene eksperimentalne doze SNPa indukuju snažan nitrozacioni i oksidacioni stress u ovim ćelijama.

Primljeno 5. maja 2004.
Odobreno 10. maja 2004.