BioNanoSci.
DOI 10.1007/s12668-016-0229-6

Nitric Oxide Production in the Rat Spinal Cord, Heart, and Liver After Spinal Cord Injury

Guzel G. Yafarova[1,2,3] · **Vyatcheslav V. Andrianov**[1,3] · **Ramil Kh. Yagudin**[2] · **Ilgiz I. Shaikhutdinov**[2] · **Khalil L. Gainutdinov**[1,3,4]

Abstract It has been shown that after 5 h of spinal cord injury, there is a decrease of nitric oxide (NO) production in the spinal cord. Seventy-two hours after the spinal cord injury, the level of NO production in the spinal cord and the heart increases by 2.5 times, and in the liver, it increases threefold. In the chronic period of a traumatic spinal cord disease in the spinal cord tissue, the level of NO production was significantly higher than at the control level.

Keywords Nitric oxide · EPR spectroscopy · Spinal cord · Heart · Spinal cord injury · Rat

1 Introduction

Nitric oxide (NO) is known as one of the most important signaling molecules regulating physiological functions of the body and cell metabolism [1–4]. NO acts as a neuromodulator in the central nervous system (CNS) and in the neuromuscular synapses, and at first, it was isolated from the vascular endothelium as a vasodilation factor. In addition to the vasodilation, NO participates in reactions of an oxidative stress, a glutamate-calcium cascade, and an inflammation [5–7]. There is a debate about the role of NO in the development of spinal cord injury (SCI); however, research on the dynamics of NO production at different stages of a traumatic disease of the spinal cord (SC) is limited [8–11]. Presumably, the role of NO depends on the concentration range, the cell type source, and the environment in which NO was received.

✉ Khalil L. Gainutdinov
kh_gainutdinov@mail.ru

[1] Institute of Fundamental Medicine and Biology of Kazan Federal University, Kazan, Russia

[2] Republican Clinical Hospital of the Tatarstan Republic, Kazan, Russia

[3] Zavoisky Physical-Technical Institute of Russian Academy of Sciences, Kazan, Russia

[4] Laboratory of Neurobiology, Institute of Fundamental Medicine and Biology, Kazan Federal University, Kazan, Russia

2 Methods

We used a model of SCI at the level of the first lumbar vertebra (L1) according to the modified method of A. Allen [12]. We studied the tissue samples of the SC, the liver, and the heart in intact animals and at different periods of the SC traumatic disease by means of the electron paramagnetic resonance (EPR) spectroscopy using the method of spin traps [13–15]. As spin traps, the complex of Fe^{2+} with diethyldithiocarbamate (DETC) was applied, leading to the formation of a stable radical $(DETC)_2$-Fe^{2+}-NO, which is characterized by the easily recognizable EPR spectrum with g-factor, $g = 2.038$, and the triplet hyperfine structure. The records of the prepared samples were carried out on the ER 200E SRC EPR spectrometer and Bruker EMX-plus X-band EPR spectrometer. The number of the paramagnetic particles in the sample was determined by comparison with a measurement standard of the known concentration. The statistical data processing was performed using the nonparametric Mann-Whitney *U* test and Student's *t* test.

3 Results and Discussion

In the intact rats, the production of NO in the SC tissues was on the average 1.3 nm/g × h, 5.2 nm/g × h in the heart, and 9.1 nm/g × h in the liver. Five hours after the injury, there is a decrease

Published online: 10 August 2016

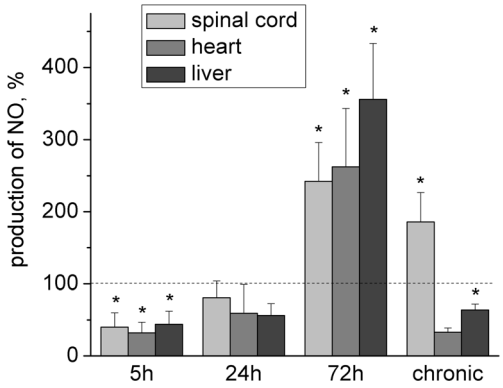

Fig. 1 The intensity of EPR signal of the complex of spin trap with NO $(DETC)_2$-Fe^{2+}-NO (in relative units) from the spinal cord, heart, and liver of rats after the spinal cord injury in different times after the injury (5 h and 72 h) and in the chronic period (more than 3 months). *The reliable difference ($p < 0.05$) versus the control group

of the NO production in the tested tissues, and it remains low up to 1 day (Fig. 1). Seventy-two hours after the injury, the level of NO production in the SC increases up to a level of 3.1 nm/g × h, and there is a significant increase in the level of NO production in the heart (up to 13 nm/g × h), and in the liver (32.5 nm/g × h). In the chronic period of the SC traumatic disease, a decrease in the level of NO production was observed in the tissues of the heart and liver, while in the heart, the level of NO production was 2.5 times less than normal, and in the liver, it made up 66 % of the control level (Fig. 1). However, in the SC tissue, the level of NO production in the chronic period of the traumatic disease was significantly higher than at the control level. There is a debate about the role of NO in the development of SCI; however, research on the dynamics of NO production at different stages of a traumatic disease of the SC is limited [8–11]. It has been demonstrated that it can exert both protective and detrimental effects in several disease states of the central nervous system, including spinal cord injury [10]. So, it is shown that inhibition of the neuronal NOS is important for neuroprotection, and the disturbances in the motor function following the SCI are associated with the SC pathology state [8]. Early NO was measured directly after the SCI in rats by the ESR spin-trapping technique using Fe^{2+} and DETC, and it demonstrated that NO formation by the constitutive NO synthase has a protective effect against cellular damage resulting from ischemia-reperfusion after the SCI, and that NO induced by the inducible NO synthase (iNOS) may be neurotoxic in the subacute phase after SCI [16]. The findings of other researchers suggest that NO generated by the iNOS of macrophages, neurons, oligodentrocytes, and astrocytes plays an important role for the acute secondary SCI that results from the apoptotic cell death [9]. It is concluded that a greater understanding of NO changes after a SC trauma, that is essential for the possibility of targeting this pathway therapeutically [11]. Thus, the dynamics of the intensity of NO formation after the SCI indicates its possible role as an inducer of apoptosis in the tissue of the damaged SC and generalized activation of NO-ergic stress-limiting system in the early stages of a SC traumatic disease.

Acknowledgments This work was funded by the subsidy of the Russian Government to support the Program of Competitive Growth of Kazan Federal University among the World's Leading Academic Centers (agreement No.02.A03.21.0002).

References

1. Ignarro, L. J., Buga, G. M., Wood, K. S., Byrns, R. E., Chaudhuri, G. (1987). Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proceedings of the National Academy of Sciences of the United States of America, 84*, 9265–9269.
2. Vanin, A. F. (1998). Dinitrosyl iron complexes and S-nitrosothiol—two possible forms of stabilization and transport of nitric oxide in biological systems. *Biohimia (in Russian), 63*, 924–938.
3. Boehning, D., & Snyder, S. H. (2003). Novel neural modulators. *Annual Review of Neuroscience, 26*, 105–131.
4. Sitdikova, G. F., & Zefirov, A. L. (2006). Gaseous messengers in nerve system Rossiiskii fizioilogicheskii zurnal imeni I.M. *Sechenova, 92*, 872–882.
5. Zamora, R., Vodovotz, V., Billiar, T. R. (2000). Inducible nitric oxide synthase and inflammatory diseases. *Molecular Medicine, 6*, 347–373.
6. Bredt, D. S. (2003). Nitric oxide signaling in brain: potentiating the gain with YC-1. *Molecular Pharmacology, 63*, 1206–1208.
7. Tomomi, G., & Masataka, M. (2006). Nitric oxide and endoplasmic reticulum stress arteriosclerosis. *Thrombosis and Vascular Biology, 26*, 1439–1446.
8. Sharma, H. S., Badgaiyan, R. D., Alm, P., Mohanty, S., Wiklund, L. (2005). Neuroprotective effects of nitric oxide synthase inhibitors in spinal cord injury-induced pathophysiology and motor functions: an experimental study in the rat. *Annals of the New York Academy of Sciences, 1053*, 422–434. doi:10.1196/annals.1344.037.
9. Kwak, E. K. (2005). The role of inducible nitric oxide synthase following spinal cord injury in rat. *Journal of Korean Medical Science, 20*, 663–669.
10. Conti, A., Miscusi, M., Cardali, S., Germano, A., Suzuki, H., Cuzzocrea, S., et al. (2007). Nitric oxide in the injured spinal cord: synthases cross-talk, oxidative stress and in ammation. *Brain Research Reviews, 54*, 205–218. doi:10.1016/j.brainresrev.2007.01.013.
11. Marsala, J., Orendácová, J., Lukácová, N., Vanický, I. (2007). Traumatic injury of the spinal cord and nitric oxide. *Progress in Brain Research, 161*, 171–183.
12. Yafarova, G. G., Tumakaev, R. F., Khazieva, A. R., Baltina, T. V. (2014). Influence of local hypothermia on H- and M- responses after contusia spinal cord injury in dogs. *Biofizika, 59*, 1017–1022.
13. Mikoyan, V. D., Kubrina, L. N., Serezhenkov, V. A., Stukan, R. A., Vanin, A. F. (1997). Complexes of Fe^{2+} with diethyldithiocarbamate or N-methyl-D-glucamine dithiocarbamate as traps of nitric oxide in animal tissues. *Biochimica Biophysica Acta, 1336*, 225–234.
14. Gainutdinov, K. L., Andrianov, V. V., Iyudin, V. S., Yurtaeva, S. V., Jafarova, G. G., Faisullina, R. I., et al. (2013). EPR study of nitric oxide production in rat tissues under hypokinesia. *Biophysics, 58*, 203–205. doi:10.1134/s0006350913020073.
15. Gainutdinov, K. L., Gavrilova, S. A., Iyudin, V. S., Golubeva, A. V., Davydova, M. P., Jafarova, G. G., et al. (2011). EPR study of the intensity of the nitric oxide production in rat brain after ischemic stroke. *Applied Magnetic Resonance, 40*, 267–278. doi:10.1007/s00723-011-0207-7.

16. Hamada, Y., Ikata, T., Katoh, S., Tsuchiya, K., Niwa, M., Tsutsumishita, Y., et al. (1996). Roles of nitric oxide in compression injury of rat spinal cord. *Free Radical Biology & Medicine, 20*, 1–9.