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Changes in the nitric oxide level in the rat liver as a response to brain injury

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ABSTRACT

Liver disturbances stimulate inflammatory reaction in the brain but little is known if injury to the brain can significantly influence liver metabolism. This problem is crucial in modern transplantology, as the condition of the donor brain seems to strongly affect the quality (viability) of the graft, which is often obtained from brain-dead donors, usually after traumatic brain injury. Because nitric oxide is one of the significant molecules in brain and liver biology, we examined if brain injury can affect NO level in the liver. Liver samples of Wistar rats were collected and studied with EPR NO-metry to detect NO level changes at different time points after brain injury. Shortly after the trauma, NO level in the liver was similar to the control. However, later there was a significant increase in the NO content in the livers starting from the 2nd day after brain injury and lasting up to the 7th day. It seems that the response to a mechanical brain injury is of the systemic, rather than local character. Therefore brain metabolism disturbances can influence liver metabolism at least by stimulating the organ to produce NO.

Introduction

Nitric oxide reveals a wide spectrum of biological action. Its beneficial or detrimental role is determined by its quantity [1,2], and in the injured brain partially depends also on the lesion type and extension [3,4]. However, brain injury causes a sequence of cellular reactions not only in the lesion site [4,5], but also in other organs outside the nervous system [6–8]. This problem has an essential significance to modern transplantology which uses organs from dead or coma donors. Various data suggest the possibility of a brain-death-induced deterioration of graft viability in liver and kidney transplants [9–11].

Many attempts have been made to monitor NO production in different organs, like brain, blood, kidney, liver, heart, and lung [12–15]. Griess and cGMP assay methods widely used for NO determination do not always reflect its concentration because of their poor specificity for blood NO [16]. Highly sensitive electron paramagnetic (spin) resonance (EPR, ESR) spectroscopy was successfully used for NO detection in various animal models [12,13,15,17]. It is based on the assumption that short living NO radical forms in situ stable NO-Fe$(DETC)_2$ spin adducts, also called mononitrosyl-iron complexes (MNIC), with iron II chelated by diethyldithiocarbamate (DETC). This very efficient NO spin-trapping system can be easily monitored by EPR [18].

It is well known that liver injury during sepsis or other pathologies induces considerable NO production. In previous studies we confirmed that a challenge with lipopolysaccharide (LPS) leads to enlarged, though quantitatively and kinetically variable NO production in the liver of adult as well as new-born animals [12]. Changes of EPR-detectable NO level may, therefore, reflect liver metabolism after LPS regarding also the developmental and physiological context.

Liver transplantation is considered to be the treatment of choice to cure many end-stage liver diseases. Pathological changes in the liver are of various origins but they usually affect nervous system [19,20]. Neurodegenerative and functional changes in the brain resulting from liver pathologies [20–22] and manifested by mental disorders are commonly named hepatic encephalopathies. During severe liver impairment, toxic substances normally modified or purified and finally removed by the liver (e.g. ammonia), accumulate in the blood and impair brain cells function. It leads to a wide spectrum of changes in the cognitive functions from subtle deficits in higher mental functions, seizures, lethargy, and depressed consciousness to coma and, when untreated, death.

On the contrary, a reverse effect of brain injury affecting liver metabolism is also possible [8,23,24]. It is however interesting that some studies did not show any changes in overall nitric oxide

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production in brain-death rats [25] while other authors [9] found that slices of liver from brain-dead rats produce significantly increased amounts of NO. This discrepancy may originate from different duration of the experiments which lead us to formulate the main aim of our study: to check whether focal brain injury has any influence on liver nitric oxide synthesis manifested by changes in the EPR spectra of spin-trapped nitric oxide.

Materials and methods

Animals and reagents

All the experimental procedures were performed in accordance with the guidelines of the Bioethical Committee at the Jagiellonian University, Krakow. Thirty-days-old Wistar rats from the local animal facility were maintained under controlled conditions at the 12-h day/night regime, water and food ad libitum.

Chemicals were obtained from Sigma–Aldrich, St. Louis, MO, USA.

Brain injury

All the described interventions were performed in deep chloral hydrate anaesthesia (400 mg/kg body weight). The injury to the brain was performed as described earlier [17,26]. Briefly, the head skin was disinfected and cut longitudinally and the skull was exposed. The injury to the left hemisphere of the brain (coordinates: 0.80 mm caudal from bregma and 2.5 mm lateral to bregma [27]) was performed using a rotating dental drill (diameter 1.4 mm, depth 2.6 mm below the skull surface) and lesion size was reproducible [17]. After injury the skin was sewed up and the animal was left to awakening.

Endotoxaemia

Earlier studies [12] indicated that during septic shock a rapid and huge NO production and secretion can be noted. To exclude the possibility that the observed changes of the NO level in the liver are a result of infections originating in the injured brain, we also decided to employ the endotoxaemia model (i.p. application of LPS, *Escherichia coli*, serotype 026:B6, in the moderate dose of 10 mg/kg body mass, see e.g. [13,14]) to compare NO production in both pathologies (brain injury and sepsis).

EPR spectroscopy

Animals were administered the spin trap at approximate volume of 0.5 ml dependent on actual body mass (DETC – 500 mg/kg body mass, in PBS, i.p.), and chelated iron II complexes ($FeSO_4 \times 7H_2O$, 50 mg/kg plus sodium citrate – 250 mg/kg body mass, s.c. [17,28]) 2, 6, 12, 24, 48, 72 h after brain injury (h.p.i.) or 7 days (d.p.i.) ($n \leqslant 4$), or 6 h and 7 days following LPS injection. A similar procedure was carried out with the control, non-injured animals. Thirty minutes after application of the spin trap the animals were deeply anaesthetised and transcardially perfused with saline to rinse out blood from tissues.

Sampling

Thirty minutes after application of the spin trap the perfused tissues were collected to further research. Right liver lobes, blood samples collected from the left heart ventricle before perfusion and brain samples were placed in glass tubes (inner diameter ca. 4 mm) and frozen in liquid nitrogen immediately after euthanasia, for analysis.

EPR assay and statistics

EPR measurements were performed in liquid nitrogen (77 K) at X-band (ca. 9.2 GHz) on an ESP300E Brucker spectrometer (liver and brain samples; field 3330 ± 250 Gs, modulation amplitude 5.26 Gs, conversion time 81.92 s, time constant 20.48 ms, microwave power 16 mW, receiver gain 1×10^4–1×10^5, scan time 83.886 s, resolution 1024 points) and a Varian E-3 spectrometer (additional evaluation of blood samples; magnetic field 3280 ± 250 Gs; modulation amplitude 10 Gs, filter time constant 0.1 s, 1–3 scans averaged, scan time 200 s, resolution 1024 points, microwave power 4 mW; amplification 5–10 $\times 10^4$).

Received EPR spectra were analyzed with WinEPR software (Brucker WINEPR system ver. 2.11, Brucker-Franzen Analytik GmbH, Germany). The peak-to-peak amplitude of the 3rd (high-field) hyperfine component of the MNIC signal (Fig. 1) was used to estimate the level of the nitrosyl-iron complexes, and indirectly – NO in the measured material [12,13,17,28]. Amplitude of each signal was normalized according to constant sample mass. The quantitative results were expressed as mean EPR signal amplitudes of at least four animals.

Our study was double-blind: neither the person who carried out EPR measurements, nor the one who elaborated the spectra later on and measured the amplitudes, did know the actual denomination of the samples. As the supposition on the normal distribution of the results cannot be maintained due to heterogeneity and putative variability of the NO-generating cell population in liver, statistical analysis were undertaken using a non-parametric Mann–Whitney *U* test.

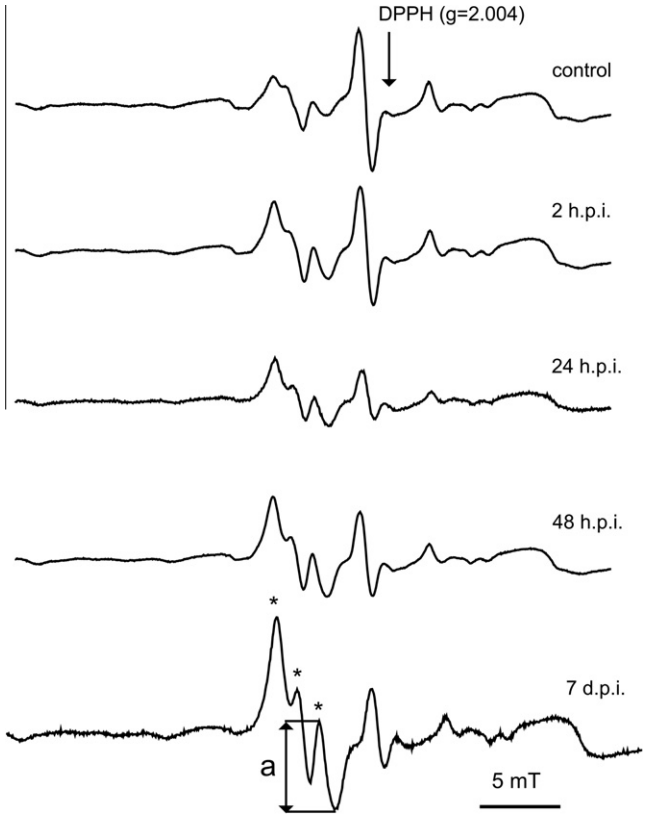

Fig. 1. Representative EPR spectra from control and experimental livers as stated on the right. Signal of MNIC adducts ($g_\perp = 2.035$, $g_{II} = 2.02$ [28,31,61]) can be appreciated in all livers. "a" – the 3rd, high field line (analytical) of the hyperfine splitting at $g_\perp$ ($A^N = 1.3$ mT [28,31,61]) ("$*$ – constituents of the hyperfine splitting, DPPH, the position of the free radical standard (1,1,diphenyl-2-picrylhydrazyl) signal ($g = 2.0037 \pm 0.0002$ [62]) Parameters of assay – see "Material and Methods". The spectra were recorded at receiver gain 10,000.