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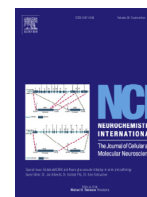

The dimethylarginine (ADMA)/nitric oxide pathway in the brain and periphery of rats with thioacetamide-induced acute liver failure: Modulation by histidine

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ABSTRACT

Hepatic encephalopathy (HE) is related to variations in the nitric oxide (NO) synthesis and oxidative/nitrosative stress (ONS), and asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthases (NOSs). In the present study we compared the effects of acute liver failure (ALF) in the rat TAA model on ADMA concentration in plasma and cerebral cortex, and on the activity and expression of the ADMA degrading enzyme, dimethylarginine dimethylaminohydrolase (DDAH), in brain and liver. ALF increased blood and brain ADMA, and the increase was correlated with decreased DDAH activity in both brain and liver. An i.p. administration of histidine (His), an amino acid reported to alleviate oxidative stress associated with HE (100 mg/kg b.w.), reversed the increase of brain ADMA, which was accompanied by the recovery of brain DDAH activity (determined *ex vivo*), and with an increase of the total NOS activity. His also activated DDAH *ex vivo* in brain homogenates derived from control and TAA rats. ALF in this model was also accompanied by increases of blood cyclooxygenase activity and blood and brain TNF-α content, markers of the inflammatory response in the periphery, but these changes were not affected by His, except for the reduction of TNF-α mRNA transcript in the brain. His increased the total antioxidant capacity of the brain cortex, but not of the blood, further documenting its direct neuroprotective power.

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1. Introduction

Hepatic encephalopathy (HE) is a complex neurological syndrome in which brain dysfunction is mainly associated with neurotoxic effects of ammonia accompanied by inflammatory response (Albrecht and Jones, 1999; Coltart et al., 2013; Felipo and Butterworth, 2002). Pathogenic action of ammonia is associated with increased nitric oxide (NO) synthesis, usually recorded as enhanced accumulation of cGMP (Hermenegildo et al., 2000; Hilgier et al., 2004). NO synthesis and subsequent accumulation of cGMP in the brain decrease on prolonged exposure to ammonia, and decreased cGMP is being considered as a key cause of cognitive dysfunction and memory impairment associated with chronic HE (Erceg et al., 2005a, 2005b). On the other hand, ammonia and inflammatory cytokines accumulating in excess in blood and brain of HE patients cooperate in inducing oxidative/nitrosative stress (ONS), making use of increased NO synthesis in generating reactive oxygen/nitrogen species, including peroxynitrite (Görg et al., 2013). Of note, nitrotyrosine, a product of reaction of peroxynitrite with tyrosine, is a good marker for the neurological alterations in HE (Montoliu et al., 2011).

Asymmetric and symmetric dimethylarginine (ADMA, SDMA) (N^G,N^G) are naturally occurring modulators of NO synthesis in mammalian tissues (Teerlink et al., 2009). ADMA can inhibit nitric oxide synthases (NOSs) that generates NO, and similarly to SDMA, can compete for cationic amino acid transporters (CAT), that supply intracellular NOS with L-arginine from plasma. ADMA is derived from the proteolysis of methylated arginine residues on various proteins. The methylation is carried out by a group of enzymes referred to as protein-arginine methyl transferases (PRMTs) (reviewed in Fackelmayer, 2005). Upon proteolysis of methylated proteins, free methylarginines are released and can then be metabolized to citrulline (Cit) through the activity of dimethylarginine dimethylaminohydrolases (DDAH-1 or DDAH-2) (Teerlink et al., 2009).

A recent study demonstrated an increase of ADMA in rat brain in a chronic HE model, suggesting the effect to be one of the causes of impaired NO synthesis in HE (Balasubramaniyan et al., 2012). On the other hand, elevated ADMA levels found in blood of human cirrhotic patients correlated well with cognitive symptoms and brain biochemical abnormalities typical for HE (Bajaj et al., 2013). However,

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the relative contribution of the ADMA-related metabolic processes in brain and peripheral tissues to the accumulation of ADMA in brain have not been analyzed so far. In the present study we measured simultaneously the concentrations of ADMA in brain and plasma of rats with acute liver failure (ALF) induced with thioacetamide (TAA). In the same rats we compared DDAH expression and activity in brain and liver. Further, contribution of inflammatory parameters, which are thought to be critical in triggering ADMA formation (Mookerjee et al., 2007a, 2007b), were evaluated by measuring TNF-α level in brain and plasma, and plasma cyclooxygenase activity. Finally, total brain NOS activity was measured as ADMA target.

Balasubramaniyan et al. (2012) succeeded in attenuating the increase of ADMA in the chronic HE model by administration of a novel ammonia-eradicating agent – ornithine phenylacetate (OP). We attempted to modulate HE-induced changes in the accumulation of ADMA and inflammatory response using L-histidine (His). His is capable of reducing oxidative stress (OS) in both the CNS and peripheral tissue (Wade and Tucker, 1998). In the CNS, His has been reported to ameliorate OS evoked by TAA-induced HE by multiple, mutually not exclusive mechanisms including inhibition of mitochondrial permeability transition (mPT) related by controlling excessive mitochondrial Gln uptake (Rama Rao et al., 2010), or preventing a decrease of glutathione content in mitochondria (Ruszkiewicz et al., 2013). We speculated that the OS-attenuating activity of His may result in correcting the ADMA/DDAH system imbalance and therefore NO synthesis. To evaluate the relative effectiveness of His as an antioxidant in the brain and in the periphery, we compared its effects on the total antioxidant capacity in brain and plasma.

2. Materials and methods

2.1. Acute liver failure model

Adult male Sprague–Dawley rats (180–220 g) were reared under standard conditions at the local animal facility. The animals had free access to food and water. All experiments were performed with agreement of local animal ethical committee that approved the experimental design. HE was induced by 3 i.p. injections of thioacetamide (TAA) (300 mg/kg b.w.) at 24 h intervals (Hilgier and Olson, 1994) and sacrificed 24 h after third injection. Control rats were administered i.p. with sodium saline solution analogically. L-histidine (His) (100 mg/kg b.w.) was injected i.p. 1 h before each TAA administration.

2.2. Dimethylarginines and amino acid determination in cerebral cortical homogenates and plasma

A 3-mL blood sample was collected and immediately plasma was separated in a centrifuge at 4 °C for 10 min, and then stored at −80 °C for subsequent analysis. Plasma ADMA concentration was measured using ADMA ELISA Kit (Immun Diagnostic) in accordance to the manufacturer protocol. For brain ADMA measurement, 100 mg of tissue was homogenized in 6% ice-cold per chloric acid and centrifuged at 12,000 rpm at 4 °C for 10 min. Supernatant was neutralized with 2M K_2CO_3, centrifuged and lyophilized. The lyophilizate was resolved in 40 mM acetate buffer and loaded onto column packed with AG50W-X8, previously conditioned with 20 mM acetate buffer. The proteins, acidic and neutral amino acids and weakly basic amines, an unwanted components were rinsed off from the cartridge with 3 mL of 20 mM acetate buffer (pH 5.5), followed by 4 mL of water, 2 mL of 0.05 M ammonia, 8 mL of water, and 1 mL of methanol. L-arginine and its methylated metabolite were eluted with 3 mL of 25% aqueous ammonia–methanol (1:1, v/v) (Pi et al., 2000). Then Arg, ADMA, His and histamine were analyzed using HPLC with fluorescence detection after derivatization in a timed reaction with *o*-phthalaldehyde (OPA) plus mercaptoethanol, as described earlier (Zielińska et al., 1999). Samples (50 μL) were injected onto 150 × 4.6 mm 5 μm Hypersil ODS column, eluted with a mobile phase of 0.075M KH_2PO_4 solution containing 10% (v/v) methanol, pH 6.2 (solvent A), and methanol (solvent B). The methanol gradient was 20–70% and the elution time was 20 min.

2.3. DDAH activity assay

DDAH activity was recorded by a colorimetric assay measuring the rate of citrulline production. Brain cortex was homogenized by sodium phosphate buffer. Tissue homogenate was pre-incubated with urease for 15 min, then 100 μL (2 mg) of homogenate was incubated with 1 mM ADMA for 90 min at 37 °C. After deproteinization, the supernatant was incubated with a “color mixture” (one part of oxime reagent with two parts of antipyrine/H_2SO_4 reagent) at 60 °C for 110 min. Each sample was analyzed with a paired blank (which omitted ADMA). The absorbance was measured by spectrophotometry at 466 nm. The DDAH activity was represented as μm citrulline formation/g protein/min at 37 °C .

2.4. Real-time PCR analysis

Total RNA was isolated using TRI Reagent (Sigma-Aldrich, USA), and then 1 μg was reverse-transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Life Technologies). Real time PCR was performed with the ABI 7500 apparatus (Applied Biosystems, Life Technologies) using the MGB Taqman probe assay. Probes for TNF-α and endogenous control β-actin were purchased from Applied Biosystems (Rn 01525859-g1 and Rn 00667869-m1, respectively). The results of the analysis were calculated in relation to the β-actin product according to, and expressed by an equation ($2^{-\Delta\Delta Ct}$). CT is the threshold cycle for target amplification (Livak and Schmittgen, 2001).

2.5. Total antioxidative capacity assay

Total antioxidant capacity (TAC) was determined using an Antioxidant Assay Kit (Sigma-Aldrich, USA) based on the formation of a ferryl myoglobin radical from myoglobin and hydrogen peroxide, which oxidizes the ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) to produce a radical cation $ABTS^{+}$, a soluble green color chromogen that can be determined at 405 nm. Fresh plasma and supernatant from homogenized (1/5; w/v) and centrifuged (12,000, 15 min, 4 °C) tissue was used to analyze TAC and protein level was determined. Trolox™ (water-soluble vitamin E analog) curve was used as a standard for antioxidants’ concentration.

2.6. Cyclooxygenase activity assay

Cyclooxygenase activities in plasma were measured with a commercially available enzyme assay (rat COX Fluorescent Activity Assay Kit; Cayman, USA) according to the protocol supplied by the manufacturer. The standards and samples were incubated with hydroperoxy endoperoxide and 10-acetyl-3,7-dihydroxyphenoxazine producing the highly fluorescent compound resorufin which was determined using an excitation wavelength of 530–540 nm and an emission wavelength of 585–595 nm.

2.7. Plasma tumor necrosis factor-α (TNF-α)

Plasma tumor necrosis factor-α (TNF-α) levels were measured by flow cytometry with BD™ Cytometric Bead Array (CBA) (Becton Dickinson, USA) strictly in accordance to the manufacturer’s protocol.

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