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Research paper

Similar effect of sodium nitroprusside and acetylsalicylic acid on antioxidant system improvement in mouse liver but not in the brain

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ABSTRACT

Background: The aim of the present study was to analyze the relative antioxidant effects of acetylsalicylic acid (ASA) and sodium nitroprusside (SNP) in mouse liver and brain.
Methods: The activity of rhodanese, 3-mercaptopyruvate sulfurtransferase (MPST) and γ-cystathionase (CSE), functioning as antioxidant proteins and capable of producing H_2S, was investigated in mouse liver and brain after intraperitoneal once a day administration of sodium nitroprusside (5 mg/kg body weight) or acetylsalicylic acid (500 mg/kg body weight) continued for 5 days. The tissues were homogenized and then the obtained supernatants were used for further determinations. At the same time, the levels of sulfane sulfur, reduced and oxidized glutathione, cysteine, cystine, and cystathionine were also studied in these tissues.
Results: Both ASA and SNP show a statistically significant increase of sulfurtransferases activities in liver. The mechanism of action of sodium nitroprusside appears to consist in liberation of nitric oxide (NO), an important signaling molecule in the mammalian body. SNP also releases cyanide ions, which are converted in the liver to thiocyanate by the enzyme rhodanese and/or MPST and/or γ-cystathionase – the activities of all the enzymes were elevated in reaction to SNP. The action of γ-cystathionase is dependent upon converting cystathionine to cysteine, a precursor of the major cellular antioxidant, glutathione. Under oxidizing conditions, an increase in cystathionine β-synthase activity might indirectly result in an increase in the antioxidant glutathione level; this was reflected by the increased GSH/GSSG ratio in the liver, but not in the brain, where a trace activity of γ-cystathionase is normally detected.
Conclusion: The results of the present investigations show that ASA and SNP may stimulate the GSH-dependent antioxidant system and protect liver cells from oxidative stress. An increased activity of the H_2S-producing enzymes and the increased GSH/GSSG ratio may lead to an elevated level of H_2S, a molecule with antioxidant properties. A similar effect was not observed in the brain. In case of both sodium nitroprusside and aspirin administration, homeostasis of sulfane sulfur level was noted in both the liver and brain.

1. Introduction

Sodium nitroprusside (SNP), the inorganic compound with the formula $Na_2[Fe(CN)_5NO]\cdot 2H_2O$ is a potent vasodilator widely used as a nitric oxide (NO) donor [1]. Its administration results also in marked increase in the concentration of NO in brain [2]. This potent antihypertensive agent, though being extremely toxic its use in the treatment of hypertensive emergencies should be avoided [3]. Endothelial cells constantly release small amounts of NO, resulting in dilation of blood vessels by relaxing vascular smooth muscle cells diffusing out of the endothelial cell and into adjacent smooth muscle cells, where NO binds to the heme group of guanylate cyclase. The enzyme is activated, thus producing cGMP, which, through a cascade of protein kinases, induces smooth muscle relaxation [4]. Besides of NO cyanide ions (CN^-) are also released from SNP [5] in a molar ratio of 5 molecules of cyanide for each molecule of SNP through a nonenzymatic reaction then being metabolized in the organism to thiocyanate by the enzyme rhodanese (thiosulphate sulfurtransferase; EC 4.4.1.1)

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Abbreviations

ASA	acetylsalicylic acid, also known as aspirin
CSE	γ-cystathionase
MPST	3-mercaptopyruvate sulfurtransferase
SNP	sodium nitroprusside
GSH	reduced glutathione
GSSG	oxidized glutathione

which is present in majority of mammalian tissues. Rhodanese distribution is restricted mainly to mitochondria. Rhodanese is responsible for transfer of sulfane sulfur atoms (atoms of sulfur bound only to other sulfur atoms and so having an oxidation state 0 or −1) from various donors to acceptors. Thiocyanate (SCN^-) formation: $CN^- + S_2O_3^{2-} \rightarrow SCN^- + SO_3^{2-}$ is a detoxification reaction in which thiocyanate become two-hundredfold less toxic then cyanides. Two other sulfurtransferases, namely 3-mercaptopyruvate sulfurtransferase (MPST, EC 2.8.1.2) and γ-cystathionase (cystathionine γ-lyase, CSE, EC 4.4.1.1) take place in thiocyanate detoxification [6]. In animal cells, MPST is located in cytosol and mitochondria mainly in liver and kidney cells, while γ-cystathionase is an enzyme found in cytosol of eukaryotic cells, with its highest activity in the liver and kidney [7,8]. MPST catalyses the transfer of sulfur atom from 3-mercaptopyruvate (the single donor) to various acceptors, which often produce sulfane sulfur-containing compounds [9]. MPST in combination with cysteine aminotransferase (CAT) produces H_2S from cysteine and bound sulfane sulfur in brain [10]. CSE fulfills a role in the pathway of cysteine synthesis from methionine and - more importantly - participates in sulfane sulfur an hydrogen sulfide generation in cells [11,12].

Acetylsalicylic acid (ASA), known under its trade name of aspirin, is a salicylate with the capability to penetrate the blood-brain barrier [13], often used as an analgesic to relieve minor pains, as an antipyretic to reduce fever, and as an anti-inflammatory medication. Its ability to suppress the production of prostaglandins and thromboxanes results from irreversible inactivation of the cyclooxygenase required for prostaglandin and thromboxane synthesis [14,15].

We found earlier that intraperitoneal injections of aspirin in a dose of 10 mg during 5 consecutive days to BALB/c and B10.PL mice increased the concentration of endogenous hydrogen sulfide in their livers and the rise of hydrogen sulfide levels was shown also in brains of BALB/c females and B10.PL males (16), however in BALB/c brains there was no statistically significant difference. Also another study indicated ASA-induced increase in H_2S level in the mouse brain, but the decrease in the liver [17].

In the present study, the authors investigated the activities of γ-cystathionase, 3-mercaptopyruvate sulfurtransferase and rhodanese, the levels of cysteine, cystine, cystathionine, GSH and GSH/GSSG ratio in the liver and brain of ASA and SNP treated mice. The objective was to explain whether changes in the activity of the three enzymes participating in H_2S formation and changes in redox status reflected by the ratio of GSH/GSSG, in response to ASA and SNP treatment, might promote the formation of H_2S.

2. Material and methods

2.1. Sources of chemicals

The provider of Folin-Ciocialteau reagent, β-nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH), lactate dehydrogenase (LDH), pyridoxal phosphate (PLP), D,L-dithiothreitol (DTT), *N*-ethylmaleimide (NEM), sodium thiosulfate pentahydrate ($Na_2S_2O_3 \cdot 5H_2O$), sodium sulfite (Na_2SO_3), ferric nitrate nonahydrate ($Fe(NO_3)_3 \cdot 9H_2O$), sodium carbonate, formaldehyde, D,L-cystathionine, L-glutathione reduced (GSH), L-glutathione oxidized (GSSG), L-cysteine, L-cystine, bathophenanthrolinedisulfonic acid (BPDS), 2,4 dinitrofluorobenzene (DNFB) and acetonitrile was Sigma-Aldrich (Chemical Company, St. Louis, MO, USA). Potassium cyanide (KCN) was obtained from Merck (Darmstadt, Germany); sodium 3-mercaptopyruvate, trifluoroacetic acid (TFA), and 2-mercaptoethanol from Flucka Chemie GmbH. Nε-methyl-L-lysine was purchased from Bachem (Bubendorf, Switzerland). All other chemicals were of reagent grade and purchased from common commercial suppliers.

2.2. Animals

Seven week male mice BALB/c weighing ~26 g were used. Animals were housed in cages in a room at the constant temperature of 20 ± 2 °C with natural light-dark cycle. They were fed standard pellet diet with free access to water. Studies presented here have been carried out in accordance with the Declaration of Helsinki and/or with the guidelines for the care and use of laboratory animals that is accepted by properly appointed Ethics Committees for the Animal Research in Krakow (number 26/III/2009).

2.3. Experimental design

Fifteen mice were divided into 3 groups. Five animals of each experimental groups received intraperitoneal injections of SNP at a dose of 5 mg per kg of body weight (0.2 mg/mouse) during 5 consecutive days or ASA (500 mg per kg of body weight, e.g., 10 mg/mouse) also during 5 days. Control animals received the same volume of isotonic saline. Two hours after the last injections the animals were killed by cervical dislocation and livers and brains were quickly removed.

2.4. Preparation of tissue homogenates

Tissues were homogenized in a blender homogenizer in 0.1 M ice-cold phosphate buffer pH 7.5 in the following proportion: 1 g tissue per 5 ml of the buffer for 1 min (3 × 20 s) at 8000–9500 rpm. The homogenates were centrifuged for 10 min at 1600 g and then the obtained supernatants were immediately employed for the determination of protein concentration, sulfane sulfur levels and the activity of rhodanese, 3-mercaptopyruvate sulfurtransferase and γ-cystathionase. For GSH, GSSG, L-cysteine, L-cystine and cystathionine determination, tissues were homogenized at 8000–9500 rpm in ice-cold 10% PCA/1 mM BPDS (1 g tissue/3 mL solution). Following centrifugation at 1400*g* at 4 °C for 10 min, the supernatant was saved at −80 °C until further use for RP-HPLC analyses.

2.5. Biochemical investigations

In the liver and brain homogenates, the γ-cystathionase activity was determined according to Czubak et al., 2002 [18], being expressed as nmoles of 2-ketobutyrate formed during 1 min incubation at 37 °C per 1 mg protein. MPST activity was essayed according to Valentine and Frankenfeld method (1974) [19] with some modifications as described by Wróbel et al. (2004) [6]. The enzyme units were defined as μmoles of pyruvate formed during 1 min at 37 °C per 1 mg protein. Rhodanese activity was assayed by Sörbo (1955) [20] method. The enzyme units were defined as