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Arsenite stimulates glutathione export and glycolytic flux in viable primary rat brain astrocytes

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ABSTRACT

Intoxication with inorganic arsenicals leads to neuropathies and impaired cognitive functions. However, little is known so far on the cellular targets that are involved in the adverse effects of arsenite to brain cells. To test whether arsenite may affect neural glucose and glutathione (GSH) metabolism, primary astrocyte cultures from rat brain were used as a model system. Exposure of cultured astrocytes to arsenite in concentrations of up to 0.3 mM did not compromise cell viability during incubations for up to 6 h, while 1 mM arsenite damaged the cells already within 2 h after application. Determination of cellular arsenic contents of astrocytes that had been incubated for 2 h with arsenite revealed an almost linear concentration-dependent increase in the specific cellular arsenic content. Exposure of astrocytes to arsenite stimulated the export of GSH and accelerated the cellular glucose consumption and lactate production in a time- and concentration-dependent manner. Half-maximal stimulation of GSH export and glycolytic flux were observed for arsenite in concentrations of 0.1 mM and 0.3 mM, respectively. The arsenite-induced stimulation of both processes was abolished upon removal of extracellular arsenite. The strong stimulation of GSH export by arsenite was prevented by MK571, an inhibitor of the multidrug resistance protein 1, suggesting that this transporter mediates the accelerated GSH export. In addition, presence of MK571 significantly increased the specific cellular arsenic content, suggesting that Mrp1 may also be involved in arsenic export from astrocytes. The data observed suggest that alterations in glucose and GSH metabolism may contribute to the reported adverse neural consequences of intoxication with arsenite.

1. Introduction

Inorganic arsenic species are toxic and considered as environmentally hazardous substances (Hughes et al., 2011; Wilcox, 2013). Such compounds are a major concern as arsenicals contaminate the ground and drinking water in many countries around the globe (Gong et al., 2014; Kim et al., 2011; Rodriguez-Lado et al., 2013). Chronic exposure to arsenicals has been associated with diabetes mellitus, black-foot disease, liver and kidney cancer as well as skin lesions (Vahidnia et al., 2007). In addition, arsenicals have been shown to cause peripheral neuritis, sensory neuropathy, encephalopathy, compromised cognitive development and impairments in learning, memory and concentration (Edwards et al., 2014; O'Bryant et al., 2011; Rodriguez et al., 2003; Roy et al., 2011; Vahidnia et al., 2007).

Presence of arsenite in drinking water leads to the accumulation of arsenicals in the brain of rodents (Jing et al., 2012; Liu et al., 2012; Wang et al., 2011; Yen et al., 2011). As astrocytic endfeet almost completely cover the brain capillaries (Mathiisen et al., 2010), arsenite that will enter the brain from the blood stream will first be encountered by astrocytes. Astrocytes have a large number of important functions in the brain (Parpura et al., 2012) which include the protection of other brain cells against the toxicity of metals and xenobiotics (Hohnholt and Dringen, 2013; Scheiber and Dringen, 2013; Schmidt and Dringen, 2012). In addition, astrocytes are considered as key players in the energy metabolism of the brain and have been suggested to provide neurons with

Abbreviations: ANOVA, analysis of variance; EGTA, ethylene glycol-bis(2-aminoethylether-)N,N,N′,N′-tetraacetic acid; GSH, glutathione; GSSG, glutathione disulfide; GSx, total glutathione; H33342, Hoechst 33342; IB, incubation buffer; LDH, lactate dehydrogenase; Mrp1, multidrug resistance protein 1; PBS, phosphate-buffered saline; PI, propidium iodide; SD, standard deviation.

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substrates for energy production (Belanger et al., 2011; Dienel, 2012; Hirrlinger and Dringen, 2010).

Astrocytes are also considered to supply neurons with precursors for glutathione (GSH) synthesis (Aoyama and Nakaki, 2013; Hirrlinger and Dringen, 2010). The tripeptide GSH is involved in the elimination of peroxides by glutathione peroxidases and is a substrate for the detoxification of xenobiotics by glutathione-S-transferases (Dringen et al., 2005; Schmidt and Dringen, 2012). GSH is also involved in the metabolism of arsenicals and has been suggested to form complexes with arsenite that are exported from cells as a mode of arsenite detoxification (Leslie, 2012; Watanabe and Hirano, 2013).

Arsenite has been reported to affect, at least in culture, properties and functions of astrocytes. In low micromolar concentrations arsenite increases the synthesis of GSH in cultured astrocytes (Sagara et al., 1996) and induces the synthesis of heat shock proteins (Catanzaro et al., 2010; Fauconneau et al., 2002). However, arsenite has also been reported to lower the expression of glutamate transporters and of glutamine synthetase in astrocytes (Zhao et al., 2012), to lower the cellular GSH content (Jing et al., 2012; Meyer et al., 2013), to compromise cell viability and to cause DNA damage (Catanzaro et al., 2010; Jin et al., 2004; Koehler et al., 2014; Zhao et al., 2012).

In order to investigate in detail the consequences of an acute arsenite exposure on the astrocytic glucose and GSH metabolism, we exposed primary rat astrocyte cultures to arsenite. Here we report that viable cultured astrocytes efficiently accumulate arsenite and that the arsenite-treatment caused a time- and concentration-dependent but reversible stimulation of glycolytic flux and GSH export. Such arsenite-induced disturbances of glucose and GSH metabolism may contribute to the observed adverse effects of inorganic arsenicals on brain functions.

2. Materials and methods

2.1. Materials

Sodium meta arsenite was obtained from Sigma–Aldrich (Steinheim, Germany). Dulbecco's modified Eagle's medium and penicillin G/streptomycin sulfate solution were purchased from Gibco/Invitrogen (Darmstadt, Germany) and fetal calf serum from Biochrom (Berlin, Germany). Bovine serum albumin, NAD, NADH and NADPH were from AppliChem (Darmstadt, Germany) and the bicinchoninic acid assay kit was obtained from Thermo-Fischer (Schwerte, Germany). Glucose-6-phosphate dehydrogenase, glutamate pyruvate transaminase, glutathione reductase, hexokinase and lactate dehydrogenase (LDH) were from Roche Diagnostics (Mannheim, Germany) and argon was purchased from Linde (Munich, Germany). Other chemicals of the highest purity available were purchased from Merck (Darmstadt, Germany), Roth (Karlsruhe, Germany) or Sigma–Aldrich (Steinheim, Germany). Sterile cell culture plates and unsterile 96-well microtiter plates were purchased from Sarstedt (Nümbrecht, Germany).

2.2. Astrocyte primary cultures

Astrocyte-rich primary cultures were prepared from the brains of neonatal Wistar rats as previously described (Hamprecht and Löffler, 1985; Tulpule et al., 2014). Rats were treated according to the regulations of the authorities of the State of Bremen. The harvested cells were seeded (300,000 viable cells per well) in 1 mL culture medium (90% Dulbecco's modified Eagle's medium, 10% fetal calf serum, 1 mM pyruvate, 20 units/mL penicillin G, 20 μg/mL streptomycin sulfate) in wells of 24-well plates or in cell culture flasks (20 million cells, 175 cm^2) in 50 mL culture medium and incubated in a Sanyo (Osaka, Japan) incubator at 37 °C with 10% CO_2 in a humidified atmosphere. The culture medium was renewed every seven days and one day prior to an experiment. For experiments, confluent cultures were used at a culture age between 14 days and 28 days.

2.3. Experimental incubation of astrocytes

To test for the acute effects of arsenite on cultured astrocytes, the cells were washed twice with 1 mL pre-warmed (37 °C) incubation buffer (IB: 20 mM HEPES, 5 mM D-glucose, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5.4 mM KCl, 0.8 mM Na_2HPO_4, 145 mM NaCl, pH 7.4) and incubated for up to 6 h with 200 μL IB containing arsenite and/or other substances in the concentrations indicated in the figures or the table. To test whether the continuous presence of arsenite is required to maintain the stimulation of GSH export and glycolytic flux, astrocytes were pre-incubated without or with 0.1 mM (30 min) and 0.3 mM arsenite (60 min), respectively. Subsequently, the cells were washed twice with 1 mL pre-warmed IB and incubated for up to 2 h in presence or absence of 0.1 mM (GSH export) or 0.3 mM (lactate production) arsenite.

After the desired incubation period, media samples were collected and the cells were washed with 1 mL ice-cold phosphate-buffered saline (PBS; 10 mM potassium phosphate buffer, pH 7.4, containing 150 mM NaCl). Media samples were used for the quantification of extracellular concentrations of lactate and glucose or were used, after dilution with 1% (w/v) sulfosalicylic acid to a final concentration of 0.5%, for the quantification of the extracellular total glutathione (GSx = amount of GSH plus twice the amount of glutathione disulfide (GSSG)) or GSSG contents. The cells were either lysed directly in 500 μL ice-cold 1% sulfosalicylic acid to determine the cellular contents of GSx and GSSG or were stored frozen at −20 °C for determination of cellular arsenic and protein contents.

2.4. Determination of cell viability and protein contents

Potential loss of membrane integrity as a measure of cell viability was investigated after a given incubation of the cells with arsenite by determining the activity of the cytosolic enzyme lactate dehydrogenase (LDH) in the medium and by the staining of cell nuclei with the membrane impermeable fluorescent dye propidium iodide (PI) as previously described (Dringen et al., 1998; Scheiber et al., 2010; Tulpule et al., 2014).

To determine the protein content of astrocyte cultures, the cells were lysed in 500 μL 50 mM NaOH and the protein content was determined according to the Lowry method (Lowry et al., 1951). The protein content of isolated mitochondria was determined by the bicinchoninic acid assay kit after lysing the mitochondria with 1% (w/v) Triton X-100 for 30 min (Brandmann et al., 2013). For both protein determination methods bovine serum albumin was used as standard protein.

2.5. Quantification of cellular arsenic contents

Cellular arsenic contents were quantified by graphite furnace atomic absorption spectroscopy using a Varian (Darmstadt, Germany) AA-240Z spectrophotometer and a Varian GTA-120 graphite tube atomizer equipped with a Varian PSD-120 programmable sample dispenser as described recently (Meyer et al., 2013). The specific cellular arsenic contents were calculated by normalizing the arsenic content of a well to the protein content of the respective well. The detection limit of the method applied to quantify arsenic in cultured astrocytes is 13 pmol per 20 μL sample which represents the lowest standard concentration used for the