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Effect of acute and prolonged alcohol administration on Mg^{2+} homeostasis in cardiac cells

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ABSTRACT

Alcoholic cardiomyopathy represents a major clinical complication in chronic alcoholics. Previous studies from our laboratory indicate that acute and chronic exposure of liver cells to ethanol results in a major loss of cellular Mg^{2+} as a result of alcohol oxidation. We investigated whether exposure to ethanol induces a similar Mg^{2+} loss in cardiac cells. The results indicate that chronic exposure to a 6% ethanol-containing diet depleted cardiac myocytes of >25% of their cellular Mg^{2+} content. Acute ethanol exposure, instead, induced a time- and dose-dependent manner of Mg^{2+} extrusion from perfused hearts and collagenase-dispersed cardiac ventricular myocytes. Pretreatment with chlormethiazole prevented ethanol-induced Mg^{2+} loss to a large extent, suggesting a role of ethanol oxidation via cyP4502E1 in the process. Magnesium extrusion across the sarcolemma occurred via the amiloride-inhibited Na^+/Mg^{2+} exchanger. Taken together, our data indicate that Mg^{2+} extrusion also occurs in cardiac cells exposed to ethanol as a result of alcohol metabolism by cyP4502E1. The extrusion, which is mediated by the Na^+/Mg^{2+} exchanger, only occurs at doses of ethanol ≥0.1%, and depends on ethanol-induced decline in cellular ATP. The significance of Mg^{2+} extrusion for the onset of alcoholic cardiomyopathy remains to be elucidated.

Introduction

Magnesium (Mg^{2+}) is the second most abundant cation within mammalian cells after potassium (Romani & Scarpa, 1992), and it is highly compartmentalized within nucleus, mitochondria, and endoplasmic or sarcoplasmic reticulum (Günther, 1986; Romani & Scarpa, 1992; Wolf, Torsello, Fasanella, & Cittadini, 2003). Within each of these compartments, total Mg^{2+} concentrations ranging between 16 and 20 mM have been measured by different techniques (Günther, 1986; Romani & Scarpa, 1992; Wolf et al., 2003). Due to technical limitations, however, assessment of free versus bound Mg^{2+} partitioning within some of these compartments remains incomplete (Günther, 1986; Romani & Scarpa, 1992; Wolf et al., 2003). A sizable amount of Mg^{2+} (4–5 mM) is also present in the cytoplasm, mostly in the form of a complex with ATP, phosphocreatine, and other phospho-nucleotides (Scarpa & Brinley, 1981). As a result, cytoplasmic free Mg^{2+} concentration ($[Mg^{2+}]i$) ranges between 0.5 and 1 mM in the majority of mammalian cells, including cardiac myocytes (Romani & Scarpa, 1992; Wolf et al., 2003).

In the absence of hormonal or metabolic stimuli, cellular Mg^{2+} concentration remains relatively stable within cardiac myocytes. Following stimulation of β-adrenergic receptors by catecholamine or isoproterenol, major and rapid extrusion fluxes of Mg^{2+} across the cell sarcolemma have been observed (Romani, Marfella, & Scarpa, 1993; Romani & Scarpa, 1990; Vormann & Günther, 1987), with minimal or no changes in $[Mg^{2+}]i$ (Fatholahi, LaNoue, Romani, & Scarpa, 2000). The main mechanism responsible for Mg^{2+} extrusion across the cell membrane of cardiac myocytes has been identified as a cAMP-phosphorylated Na^+/Mg^{2+} exchanger both in intact cells (Romani et al., 1993; Romani & Scarpa, 1990; Vormann & Günther, 1987) and sarcolemmal vesicles (Cefaratti & Romani, 2007).

Clinical and experimental data indicate alcohol consumption as one of the main causes of Mg^{2+} loss from liver cells (Romani, 2008). Our group has extensively investigated the mechanisms responsible for the hepatic Mg^{2+} loss and reported that acute ethanol administration causes two distinct effects. On one hand, ethanol inhibits anaerobic glycolysis in a dose-dependent manner, transiently decreasing cellular ATP and reducing its ability to complex cytoplasmic Mg^{2+} (Tessman & Romani, 1998). The consequent

Abbreviations: 4-MP, 4-methyl-pyrazole; cyP4502E1, cytochrome P450-2E1; CMZ, chloromethiazole; FCCP, carbonyl cyanide p-trifluoromethoxy phenylhydrazone; LDH, lactic dehydrogenase; $[Mg^{2+}]i$, cytoplasmic free magnesium concentration.

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increase in cytosolic free Mg^{2+} promotes Mg^{2+} extrusion through the Na^+/Mg^{2+} exchanger (Tessman & Romani, 1998). On the other hand, ethanol inhibits the Mg^{2+} entry mechanism for more than 45 min after alcohol removal (Torres, Konopnika, Berti-Mattera, Liedtke, & Romani, 2010), *de facto* hampering the restoration of proper Mg^{2+} homeostasis. Similar effects occur on prolonged bases in hepatocytes from animals exposed to alcohol in the diet for 3 weeks (Torres, Cefaratti, Berti-Mattera, & Romani, 2009; Young, Cefaratti, & Romani, 2003).

Ethanol administration is deleterious for several tissues, including the heart, with alcoholic cardiomyopathy representing a major pathological complication in alcoholics (Lucas, Brown, Wassef, & Giles, 2005). A full understanding of ethanol deleterious effects on cardiac cells, however, is hindered by the clinical and experimental evidence that acute, moderate ethanol consumption exerts protective effects on the heart and the cardiovascular system (Lucas et al., 2005; Tolstrup, Nordestgaard, Rasmussen, Tybjærg-Hansen, & Grønbæk, 2008), in contrast to prolonged intake of high doses of ethanol, which promote the development of alcoholic cardiomyopathy (Dancy & Maxwell, 1986; Lucas et al., 2005; Tolstrup et al., 2008) and dilated cardiac hypertrophy (Dancy & Maxwell, 1986). Such a discrepancy has been explained with the dose of alcohol ingested and the way alcohol is metabolized within the cardiac myocyte. Due to the absence of alcohol dehydrogenase (EC 1.1.1.1), cardiac cells metabolize ethanol mainly through the alcohol-inducible cytochrome P4502E1 (cyP4502E1, EC 1.14.13.n7) located within the sarcoplasmic reticulum (Tolstrup et al., 2008). Like alcohol dehydrogenase, cyP4502E1 converts ethanol to acetaldehyde, and it is to this metabolite that the deleterious effects of alcohol in cardiac muscle have been attributed (Zhang, Li, Brown, & Ren, 2004). In addition, oxidation of ethanol via cyP4502E1 is associated with the production of reactive oxygen species (ROS) and free radicals, and both these moieties contribute significantly to the development of alcoholic cardiac pathology (Tolstrup et al., 2008; Zhang et al., 2004).

In the present study, using a combination of animal and cellular models, we investigated the effects of acute and chronic exposure to ethanol on cardiac Mg^{2+} homeostasis. The reported results indicate that short-term exposure to low doses of ethanol does not result in Mg^{2+} extrusion or changes in cellular Mg^{2+} content, at variance with what is observed in liver cells. In contrast, prolonged and chronic exposure to high doses of ethanol elicits a major loss of Mg^{2+} from the cells. The modality of Mg^{2+} extrusion largely resembles that observed in hepatocytes in terms of time- and dose-dependence. The effect of ethanol is markedly reduced by inhibitors of cyP4502E1, suggesting that ethanol metabolism through the cytochrome is key to induce Mg^{2+} mobilization from cardiac cells. Consistent with this observation, a significant portion of cellular Mg^{2+} is lost from the sarcoplasmic reticulum where the cyP4502E1 is located. Overall, our data indicate that loss of cellular Mg^{2+} constitutes an essential component of the response of cardiac myocytes to ethanol exposure, as already observed in liver cells. Because of the close association of Mg^{2+} with ATP within cytoplasm and mitochondria (Günther, 1986; Romani & Scarpa, 1992; Scarpa & Brinley, 1981), and its role in regulating reticular Ca^{2+} cycling (Laver & Honen, 2008), it is tempting to speculate that Mg^{2+} loss may have significant repercussions on ATP utilization and contractility within the heart.

Materials and methods

Materials

Collagenase (CLS-I) was from Worthington Biochemical Corporation (Lakewood, NJ). All other chemicals were of analytical grade (Sigma–Aldrich, St. Louis, MO).

Animal ethics

Animals were maintained and handled in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Science, National Research Council 1996), as approved by the Animal Resource Center at Case Western Reserve University, Cleveland, Ohio.

Chronic alcohol model

Male Sprague–Dawley rats (180–200 g body weight) were randomly divided into control- and ethanol-treated groups and housed individually in metabolic cages. Ethanol-treated rats were maintained for 3 weeks on a 6% (v/v) ethanol Lieber-DeCarli diet. Pair-fed control rats received an isocaloric liquid control diet (Dyets, Bethlehem, PA), as previously reported (Torres et al., 2010; Young et al., 2003). Age-matched, Purina Chow pellet-fed control rats were used for comparison. Weight gain was recorded weekly. To study recovery, the ethanol diet was suspended after 3 weeks of alcohol administration, and rats of both experimental groups were fed the liquid control diet for a period of time varying from 2 to 15 days.

Determination of total Mg^{2+} and Na^+ content

Cardiac total Mg^{2+}, Na^+, K^+, and Ca^{2+} contents were measured in hearts of animals maintained on ethanol diet and liquid control diet as previously reported (Young et al., 2003). Briefly, the hearts were explanted, quickly rinsed in ice-cold sucrose (250 mM) solution, blotted on absorbing paper, and weighed. The atria were removed and the ventricles homogenized (10% w/v) in 250 mM sucrose using a Polytron homogenizer (15-sec pulses × 3). The homogenate was acidified by addition of HNO_3 (10% final concentration), and extracted overnight. Following sedimentation of denatured proteins (10,000 rpm × 3 min), the Na^+ and Mg^{2+} contents of the acid extract were measured by atomic absorbance spectrophotometry (AAS) in a Perkin–Elmer 3100 spectrometer calibrated with appropriate standards, and normalized per mg of protein and g of tissue.

Langendorff perfusion procedure

Following exposure to Lieber-DeCarli diet or to isocaloric control diet, the animals were anesthetized by intraperitoneal (i.p.) injection of a saturated pentobarbital solution (50 mg/kg body weight). Once deep anesthesia was attained (assessed by the disappearance of pain reflex and corneal touch), the chest was opened and the heart rapidly excised at the aortic arch. The aorta was mounted on a truncated 16 gauge needle and the heart was flushed with a medium containing (mM): NaCl, 120; KCl, 3; $CaCl_2$, 1; $MgCl_2$, 0.8; K_2HPO_4, 1.2; $NaHCO_3$, 12; glucose, 15; HEPES, 10; pH 7.2 at 37 °C, equilibrated with an O_2:CO_2 (95:5, v/v) gas mixture (perfusion medium). The heart was connected to a perfusion pump and retrograde-perfused in a Langendorff manner with the medium indicated above equilibrated with O_2:CO_2 (95:5, v/v) at a flow rate of 7 mL/g/min, at 37 °C (Romani & Scarpa, 1990). After a few minutes of equilibration, the perfusion medium was switched to one having a similar composition but devoid of Mg^{2+} (Mg^{2+}-free medium). The contaminant Mg^{2+} present in the medium was measured by AAS and found to range between 5 and 7 μM. Samples of the perfusate were collected at 30 s intervals, and the Mg^{2+} content measured by AAS. The first 10 min provided a baseline for the subsequent addition of the reported ethanol doses. Ethanol was diluted directly into the perfusion medium, and administered for 10 min (Fig. 1). To estimate the total amount of Mg^{2+} extruded from