ACETALDEHYDE-INDUCED CARDIAC CONTRACTILE DYSFUNCTION MAY BE ALLEVIATED BY VITAMIN B1 BUT NOT BY VITAMINS B6 OR B12

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Abstract — Aims: Chronic alcohol exposure leads to a deficiency of group B vitamins and increased risk of alcoholic cardiomyopathy characterized by impaired ventricular contractility. This study was designed to examine the effect of group B vitamin supplementation on short-term exposure of the main alcohol metabolite acetaldehyde (ACA)-induced cardiac contractile dysfunction in rat ventricular myocytes. Methods: Mechanical contractile properties were evaluated by an IonOptix SoftEdge® system. Protein damage and apoptosis were determined by protein carbonyl and caspase-3 assays, respectively. Results: Short-term (4–6 h) culture of myocytes with ACA (10 µM) depressed peak shortening amplitude, maximal velocity of shortening/relengthening, shortened duration of shortening but not the duration of relengthening. ACA exposure also enhanced protein carbonyl formation and apoptosis in ventricular myocytes. The toxin-induced mechanical defects, protein damage and apoptosis were ablated by vitamin B1 (10 µM), an essential vitamin required for DNA synthesis and repair. Vitamin B6 (10 µM) attenuated ACA-induced impairment of shortening duration. Vitamin B12 (1 mM) attenuated ACA-induced reduction in maximal velocity of shortening/relengthening. Unlike vitamin B1, none of the other ACA-elicited alterations in myocyte mechanical function were affected by vitamin B6 or vitamin B12. Vitamin B6 and vitamin B12 partially, but significantly, attenuated the ACA-induced carbonyl formation without affecting ACA-induced apoptosis. Conclusions: These data provide evidence that vitamin B1 supplementation may be protective for ACA-induced cytotoxicity through protection against protein damage and apoptotic cell death in ventricular myocytes.

INTRODUCTION

Chronic alcohol ingestion often leads to alcoholic cardiomyopathy characterized by cardiomegaly, disruptions of myofibrillar architecture, reduced myocardial contractility and decreased ejection fraction (Preedy and Richardson, 1994; Thomas et al., 1994; Ren and Brown, 2000; Spies et al., 2001; Hintz et al., 2003). Several scenarios have been formulated for the onset of alcoholic cardiomyopathy including cardiotoxicity of alcohol or its metabolites (Preedy et al., 1999) and accumulation of fatty acid ethyl esters (Laposata and Lange, 1986). Acetaldehyde, the first oxidized metabolic product of ethanol, is a candidate toxin for the onset of alcoholic cardiomyopathy (Aberle and Ren, 2003; Hintz et al., 2003). Our laboratory showed that acetaldehyde may directly impair cardiac excitation–contraction (E–C) coupling, inhibit sarcoplasmic reticulum (SR) Ca2+ release function, promote oxidative stress, lipid peroxidation and protein damage (Ren et al., 1997, 2002; Ren and Brown, 2000; Aberle and Ren, 2003; Hintz et al., 2003). However, the precise mechanism(s) of action behind acetaldehyde and/or alcohol-induced cardiac toxicity was not fully clear.

Recent evidence suggested that depletion of vitamins as a consequence of alcohol use may play a role in alcohol-induced organ and tissue damage (Ba et al., 1996, 1999). Deficiencies in group B vitamins and folic acid following alcohol intake are among the key causative factors associated with alcoholic organ and tissue damage (Ba et al., 1999), consistent with the observation of idiopathic dilated cardiomyopathy in patients with vitamin deficiency (Whyte et al., 1982; Tobias et al., 1989). Group B vitamins are important water-soluble vitamins often obtained through diet. They are essential for DNA synthesis and repair. In addition, vitamins B6 and B12 are required for biological formation of homocysteine. The aim of this study was to investigate the influence of group B vitamin supplementation on short-term acetaldehyde exposure-induced cardiac mechanical dysfunction in isolated rat ventricular myocytes. Direct measurement of cardiomyocyte mechanics on a beat-to-beat basis is essential to the evaluation of cardiac excitation–contraction coupling under pathophysiological conditions such as alcoholism (Ren and Brown, 2000; Aberle and Ren, 2003).

MATERIALS AND METHODS

Myocyte isolation procedures

The experimental procedures described in this study have been approved by the University of North Dakota and the University of Wyoming Animal Care and Use Committees. In brief, ventricular myocytes were isolated from adult male Sprague–Dawley rats (200–225 g) by a retrograde coronary perfusion method as previously described (Aberle et al., 2003). Ventricular myocytes were dissociated under sterile conditions by collagenase (223 U/ml, Sigma Chemicals, St Louis, MO) and hyaluronidase (0.1 mg/ml, Sigma) perfused through the coronaries, and further digested by trypsin (0.02 mg/ml) during trituration (5 min) after the tissue was removed from the Langendorff perfusion apparatus and minced. The cells were maintained at 37°C in an incubator with 100% humidity and 5% CO2. Only rod-shaped myocytes with clear edges were selected for recording of mechanical properties.

Cell shortening/relengthening

Mechanical properties of ventricular myocytes were assessed using an IonOptix MyoCam® system (IonOptix Incorporation, 2004).
Milton, MA) as described (Aberle et al., 2003). In brief, cells were placed in a chamber mounted on the stage of an inverted microscope (Olympus IX-70) and superfused (~2 ml/min at 25°C) with a buffer containing (in mM): 131 NaCl, 4 KCl, 1 CaCl₂, 1 MgCl₂, 10 glucose, 10 HEPES, at pH 7.4. The cells were field stimulated to contract at a frequency of 0.5 Hz. Changes in cell length during shortening and relengthening were captured and converted to digital signal. Cell shortening and relengthening were assessed using the following indices: peak shortening (PS), time-to-PS (TPS), time-to-90% relengthening (TR90) and maximal velocities of shortening and relengthening (± dL/dt).

**Protein carbonyl assay**

To assess protein damage, the carbonyl content of protein extracted from cardiac myocytes was determined as described (Hintz et al., 2003; Ren et al., 2003). Briefly, myocyte number was counted and proteins were extracted. Nucleic acids were eliminated by treating the samples with 1% streptomycin sulfate for 15 min, followed by a 10 min centrifugation (11 000 g). Protein was precipitated by adding an equal volume of 20% TCA to protein (0.5 mg) and centrifuged for 1 min. The TCA solution was removed and the cells were resuspended in 10 mM 2,4-dinitrophenylhydrazine (2,4-DNPH) solution. Myocytes were incubated at room temperature for 20 min and were centrifuged for 3 min following addition of 500 µl of 20% TCA. The supernatant was discarded and the precipitate was resuspended in 6 M guanidine solution and centrifuged for 3 min. The maximum absorbance (360–390 nm) of the supernatant was read against appropriate blanks (water, 2 M HCl) and the carbonyl content was calculated using the molar absorption coefficient of 22 000 M⁻¹·cm⁻¹.

**Caspase-3 activation assay**

Caspase-3 plays a critical role in apoptotic signaling; induction of Bax may lead to the activation of caspase-3 (Telford et al., 1994). Ventricular myocytes were plated on 100 mm petri dishes. Caspase-3 activity was determined using the colorimetric kit purchased from R&D System (Minneapolis, MN). Myocytes were harvested and washed once with phosphate-buffered saline. After the myocytes were lysed, reaction buffer was added to the myocytes followed by the additional 5 µl of caspase-3 colorimetric substrate (DEVD-pNA) and incubated in a 96-well plate for 4 h at 37°C in a CO₂ incubator. The plate was then read with a microplate reader at 405 nm (Ren et al., 2002).

**Experimental protocols**

Cardiac myocytes were incubated with acetaldehyde (10 µM) in the presence or absence of vitamin B1 (thiamin, 10 µM), vitamin B6 (pyridoxine) or vitamin B12 (cyanocobalmin) on acetaldehyde (ACA)-induced myocyte contractile response in adult rat ventricular myocytes. Cells were incubated with ACA (10 µM) for 4–6 h in the absence or presence of vitamin B1 (10 µM), vitamin B6 (10 µM) or vitamin B12 (1 mM). (A) Representative traces from control and ACA-treated myocytes; (B) representative traces from myocytes treated with ACA in the presence of vitamin B1, B6 or B12; (C) resting cell length; (D) peak shortening (% of resting cell length). Mean ± SEM, n = 40–41 myocytes/group. *P < 0.05 vs control group; #P < 0.05 vs ACA group.
vitamin B6 (pyridoxine, 10 µM) or vitamin B12 (cyanocobalmin, 1 mM) in 20-ml sealed vials that possess silicone septa (VWR) for 4–6 h before mechanical and biochemical properties were evaluated.

Data analysis
Data were mean ± SEM. Differences between means between groups were assessed using analysis of variance (ANOVA). When an overall significance was determined, a Dunnetts post hoc analysis was incorporated. P-value <0.05 was considered significant.

RESULTS

Effect of B group vitamin supplementation on acetaldehyde-induced mechanical dysfunction in ventricular myocytes
Representative traces and pooled data depicted in Fig. 1 demonstrated that peak myocyte shortening was significantly depressed following a 4–6 h incubation of acetaldehyde (10 µM) in vials sealed with silicone septa. However, acetaldehyde-induced inhibition of myocyte shortening was prevented by co-incubation of vitamin B1 (10 µM) but was not affected by vitamin B6 (10 µM) or vitamin B12 (1 mM). Neither acetaldehyde nor group B vitamins exhibited any significant effect on resting cell length. The maximal velocities of myocyte shortening and relengthening (± dL/dt) were both depressed by acetaldehyde (10 µM) following a 4–6 h incubation in sealed vials. Consistent with its effect on myocyte peak shortening, vitamin B1 (10 µM) effectively blunted the acetaldehyde-induced depression in ±dL/dt while vitamin B6 (10 µM) had no effect. Co-incubation of vitamin B12 (1 mM) also nullified acetaldehyde-induced significant inhibition of ±dL/dt (Fig. 2A and B). Acetaldehyde significantly shortened time-to-peak shortening (TPS) while displaying no effect on time-to-90% relengthening (TR90). The acetaldehyde-induced shortening of TPS was ablated by co-incubation of vitamin B1 (10 µM) but not that of vitamin B6 (10 µM) or vitamin B12 (1 mM). TR90 was unaffected by co-treatments with any of the group B vitamins (Fig. 2C and D). Lastly, none of the vitamins tested had a significant effect on myocyte mechanics in control myocytes without acetaldehyde treatment (data not shown).

Effect of B group vitamin supplementation on acetaldehyde-induced protein carbonyl formation and caspase-3 activation in ventricular myocytes
Alcohol and its metabolite acetaldehyde have been demonstrated to lead to irreversible protein damage and apoptotic cell death (Preedy et al., 1999; Hintz et al., 2003; Ren et al., 2003). Results shown in Fig. 3 indicate that protein carbonyl formation and caspase-3 activation were both significantly elevated in...
ventricular myocytes following a 4–6 h incubation of acetaldehyde (10 µM) in vials sealed with silicone septa. However, acetaldehyde-induced elevation in both protein carbonyl formation and caspase-3 activation was prevented by co-incubation of vitamin B1 (10 µM). Co-incubation of vitamin B6 (10 µM) or vitamin B12 (1 mM) partially but significantly attenuated acetaldehyde-induced protein damage but did not affect acetaldehyde-induced caspase-3 activation. None of the vitamins had a significant effect on protein carbonyl formation and caspase-3 activation in control myocytes in the absence of acetaldehyde treatment (data not shown).

DISCUSSION

The major finding of our study indicated that short-term acetaldehyde incubation-induced impairment in cardiac contractile function, protein damage and apoptosis may be preventable by vitamin B1 supplementation. The effect is relatively selective and specific since neither B6 nor B12 were effective in completely reconciling all acetaldehyde-induced cardiac mechanical defects, protein damage and apoptotic cell death. These findings provide initial support for the hypothesis that deficiency in vitamin B1 (thiamin) following alcohol exposure may be a causal factor in cardiac complications under alcoholism.

Vitamin B1 and vitamin B6 levels are reduced in 33% and 25% of the patients, respectively, with chronic alcohol exposure. Many of these vitamin-deficient patients displayed cardiovascular dysfunction including idiopathic dilated cardiomyopathy (Whyte et al., 1982; Tobias et al., 1989). The most common forms of severe complications of vitamin B1 deficiency are beriberi and Wernicke–Korsakoff syndrome (Singleton and Martin, 2001). Similar to ethanol, acetaldehyde itself has been shown to reduce thiamin levels in blood within 12 h following intravenous injection. Thiamin levels decreased by acetaldehyde injection returned to normal levels 72 h after injection (Takabe and Itokawa, 1983). Therefore, it is plausible to speculate that an acute ethanol ingestion-induced decrease in thiamin may be due to its metabolism into acetaldehyde and subsequently, catabolism of acetaldehyde. The mechanism of action behind ethanol or acetaldehyde-induced deficiency in group B vitamin may be related to the reduced activity of thiamin pyrophosphokinase and the thiamin pyrophosphate synthetic enzyme (Rindi et al., 1986; Singleton and Martin, 2001). Thiamin administration reverses ethanol-induced cytoplasmic damage by protecting against ethanol-induced change in membrane fluidity and stability (Ba et al., 1996). This received support from our current study that vitamin B1 abolished acetaldehyde-induced protein damage and apoptotic cell death. It is plausible that interruption of membrane fluidity may serve as one of the mechanisms behind the acetaldehyde-induced cardiac mechanical and biochemical defects. Acetaldehyde has been demonstrated to inhibit the activity of a number of membrane ion transporting proteins, which contribute to alteration of the membrane fluidity (Tillotson et al., 1981). Our results observed that vitamin B6 and vitamin B12 only partially attenuated protein carbonyl formation and exhibited no effect on caspase-3 activation following acetaldehyde exposure, consistent with the minor effect of these two B group vitamins on acetaldehyde-induced cardiac mechanical dysfunctions. Although it is beyond the scope of the present study, it can be postulated that vitamin B1-elicted cardiac protection is related to antagonism against oxidative stress in acetaldehyde-mediated cell damage (Aberle and Ren, 2003).

In summary, results from our present study confirmed that short-term acetaldehyde exposure directly leads to cardiac mechanical dysfunction, protein damage and apoptosis in ventricular myocytes. More importantly, our findings suggest that acetaldehyde-induced cardiac mechanical dysfunction, protein damage and apoptotic cell death in ventricular myocytes may be prevented by vitamin B1 supplementation, but not by vitamin B6 or vitamin B12. These data indicate a relative specificity of vitamin B1 or its deficiency over other B group vitamins in acetaldehyde-induced cardiac defects and possibly onset of alcoholic cardiomyopathy. Further study is warranted to depict the role of vitamin B group deficiency, especially vitamin B1, on the propensity of alcohol-induced cardiac disorders, and the therapeutic potential of vitamin B1 (thiamin) in alcoholic complications in the cardiovascular system.

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REFERENCES


