Influence of genetically predisposed diabetes on ethanol-induced depression of cardiac contraction in adult rat ventricular myocytes

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Diabetes mellitus and alcohol (ethanol) intake are two positively correlated major risk factors for cardiovascular abnormalities. However, the interaction of the two on cardiac function is largely unknown. The purpose of the present study was to examine the impact of genetically predisposed diabetes on acute ethanol exposure-induced cardiac contractile depression at the myocyte level. Ventricular myocytes from spontaneously biobreeding diabetes-prone (BBDP) rats and their diabetes-resistant littermates (BBDR) were stimulated to contract at 0.5 Hz. Contractile properties analysed include: peak shortening amplitude (PS), time-to-PS (TPS), time-to-90% relengthening (TR$_{90}$) and maximal velocities of shortening/relengthening ($\pm dL/dt$). BBDP rats displayed hyperglycaemia, reduced body weight gain and increased cardiac, hepatic and renal size. Myocytes isolated from BBDP rat hearts exhibited prolonged TPS and TR$_{90}$ associated with normal PS and $\pm dL/dt$, compared with myocytes from the BBDR group. Acute ethanol exposure (80–640 mg dl$^{-1}$) caused a concentration-dependent inhibition of PS in both BBDR and BBDP myocytes. However, the degree of inhibition of PS was significantly reduced in BBDP myocytes compared to that of BBDR myocytes. The maximal inhibition was 52.9% and 28.4% in BBDR and BBDP groups, respectively. Ethanol significantly depressed $\pm dL/dt$ in both BBDR and BBDP myocytes. In addition, ethanol did not affect TPS or TR$_{90}$ in either the BBDR or BBDP group. Collectively, these results suggest that the ethanol-induced depression in cardiac myocyte contraction may be ‘shadowed’ by genetically predisposed diabetes. Experimental Physiology (2002) 87.3, 293–298.
linked to an increased incidence of type 2 diabetic patients after adjustment of age, body mass index, cigarette smoking and family history of diabetes (Holbrook et al. 1990; Kao et al. 2001). Ethanol may inhibit glyconeogenesis and lead to hypoglycaemia, thus making alcohol use in type 1 diabetic patients receiving hypoglycemic therapies potentially fatal (Meeking & Cavan, 1997). To date, the nature of the relationship between ethanol- and diabetes- (both types) induced cardiac myopathic dysfunction remains undefined.

Although light to moderate alcohol consumption may be beneficial in preventing cardiac events in diabetic patients, the safety concern is always debatable for diabetic patients even with moderate therapeutic alcohol intake to decrease their cardiac risk (Ajani et al. 2000). Therefore, the aim of the present study was to examine the impact of genetic diabetes on acute alcohol exposure-induced cardiac contractile depression using ventricular myocytes from spontaneously biobreeding diabetes-prone (BBDP) rats and their diabetes-resistant (BBDR) littermates. The genetically predisposed, spontaneously diabetic BB Wistar rat model displays the typical diabetic syndrome including hypo-insulinaemia, hyperglycaemia and glycosuria, potentially as a result of cell-mediated autoimmune destruction of pancreatic β-cells. As a model of diabetes that depends on insulin for survival, the BB Wistar rat is the closest counterpart to human type 1 diabetes. Distinct morphological and functional abnormalities have been reported in BB Wistar rat myocardium and myocytes, including loss of myofilaments, disruption of mitochondria, dilatation of sarcoplasmic reticulum, depressed myocardial contractility, prolonged ventricular contraction and relaxation, and impaired sarcoplasmic reticulum (SR) Ca\(^{2+}\) function (Hsiao et al. 1987; Rodrigues et al. 1990; Broderick et al. 1994; Ren & Bode, 2000).

**METHODS**

**Experimental animals**

The experiments described here were approved by the Animal Care Committee of the University of North Dakota School of Medicine. In brief, male biobreeding diabetes-prone (BBDP) and age-matched diabetes-resistant (BBDR) rats were purchased at 60 days of age from the breeding colony at the University of Massachusetts, Biomedical Research Models, Inc. The rats were housed individually and allowed access to standard laboratory rat chow and tap water *ad libitum*. Upon the onset of type 2 diabetes, indicated by glucosuria, the BBDP rats were anaesthetized with bivalent sodium (50 mg kg\(^{-1}\), i.p.) and a sustained-release insulin implant (Linplant, Linshin, Canada) was inserted in the dorsal neck region. The length of the Linplant was adjusted to control for chronic moderate hyperglycaemia in the BBDP rats. Blood glucose levels were measured using a glucose monitor (Accu-ChekII, model 792, Boehringer Mannheim Diagnostics, Indianapolis, IN, USA). The active life of the Linplant implant was approximately 40 days, thereby eliminating the need for daily insulin injections. Re-implantation was performed when blood glucose levels exceeded 350 mg dl\(^{-1}\). The animals were killed at 12 months of age.

**Isolation of cardiac myocytes**

Ventricular myocytes were isolated using the method described previously (Ren & Bode, 2000). Briefly, animals were anaesthetized with a ketamine–xylazine mixture in solution (5:3, 1.32 mg kg\(^{-1}\), i.p.). The muscle relaxant ketamine and local anaesthetic xylazine were selected (approved by the Animal Care Committee of University of North Dakota) to avoid the potential direct cardiovascular effect of anaesthetics such as pentobarbital. Hearts were rapidly removed and perfused (at \(37 ^\circ\)C) with oxygenated (5% CO\(_2\)–95% O\(_2\)) Krebs-Henseleit bicarbonate (KHB) buffer (mM: NaCl 118, KCl 4.7, CaCl\(_2\) 1.25, MgSO\(_4\) 1.2, KH\(_2\)PO\(_4\) 1.2, NaHCO\(_3\) 25, N-[2-hydro-ethyl]-piperazine-N\(^{\prime}\)-[2-ethanesulfonic acid] (Hepes) 10, glucose 11.1. Hearts were subsequently perfused with a nominally Ca\(^{2+}\)-free KHB buffer for 2–3 min followed by a 20 min perfusion with Ca\(^{2+}\)-free KHB containing 176 U ml\(^{-1}\) collagenase (Worthington Biochemical Corp., Freehold, NJ, USA) and 0.1 mg ml\(^{-1}\) hyaluronidase (Sigma Chemical, St Louis, MO, USA). After perfusion, ventricles were removed and minced, under sterile conditions, and incubated with the above enzymatic solution for 3–5 min. The cells were further digested with trypsin before being filtered through a nylon mesh (300 μm) and subsequently separated from the enzymatic solution by centrifugation (60 g for 30 s). Myocytes were resuspended in a sterile filtered, Ca\(^{2+}\)-free KHB buffer containing (mM): NaCl 131, KCl 4, MgCl\(_2\) 1, Hepes 10, and glucose 10, supplemented with 2% bovine serum albumin, with a pH of 7.4. The mechanical properties of myocytes remained stable for 24 h. Cell were not used if they had any obvious sarcosomal blebs or spontaneous contractions.

**Cell shortening/relengthening**

Mechanical properties of ventricular myocytes were assessed using an IonOptix MyoCam system (IonOptix Corp., Milton, MA, USA) (Ren & Bode, 2000). In brief, cells were placed in a chamber mounted on the stage of an inverted microscope (Olympus IX-70) and superfused (~1 ml min\(^{-1}\) at 37 °C) with a buffer containing (mM): NaCl 131, KCl 4, CaCl\(_2\) 1, MgCl\(_2\) 1, glucose 10, Heps 10, and pH 7.4. The cells were field stimulated at frequency of 0.5 Hz, 3 ms in duration. A video-based edge detector was used to capture and convert changes in cell length during shortening and relengthening into an analog voltage signal. Cell shortening and relengthening were assessed using the following indices: peak shortening amplitude (PS), time-to-PS (TPS) and time-to-90% relengthening (TR\(_{90}\)), maximal velocities of shortening/relengthening (± dL/dt).

**Experimental protocols**

Myocytes were first allowed to contract at a stimulation frequency of 0.5 Hz for 5 min to ensure steady state (myocytes with rundown > 10% were not studied further) before perfusing with ethanol (80–640 mg dl\(^{-1}\)) containing contractile buffer. A 5 min interval was allowed between the ethanol doses. After the last ethanol dose (640 mg dl\(^{-1}\)), the cells were washed with normal contractile buffer for 5 min.

**Data analysis**

For each experimental series, data are presented as means ± s.e.m. Differences in means between groups were assessed using Students *t* test (used for Table 1), whereas within group comparisons between mean values were calculated by repeated measures analysis of variance (ANOVA, used for all figures). When an overall significance was determined, Dunnett’s *post hoc* analysis was used.
RESULTS

General features of BBDR and BBDP rats

The sustained diabetic state significantly reduced body weight gain and elevated plasma glucose levels in BBDP rats compared to the age-matched BBDR littermates. There was no difference in absolute heart weights, liver or kidney weights (data not shown), between BBDR and BBDP groups. The relative sizes of heart, liver and kidney (organ-to-body weight ratio) were all significantly greater in the BBDP group than in the BBDR group (Table 1).

Baseline mechanical properties of BBDR and BBDP myocytes

The average resting cell length (CL) of ventricular myocytes isolated from the BBDR and the BBDP animals was $127.1 \pm 6.3 \mu m \ (n=8)$ and $142.7 \pm 7.7 \mu m \ (n=9)$, respectively ($P > 0.05$, one averaged CL was used from each rat). The peak shortening amplitude (PS) was not significantly different between the BBDR ($6.62 \pm 1.24\%\ CL$, $n=8$ rats) and the BBDP ($5.94 \pm 0.55\%\ CL$, $n=9$ rats) groups. Myocytes from the BBDP group exhibited a prolonged duration of both shortening (TPS) and relengthening (TR90) compared to their BBDR counterparts (Fig. 1A and Fig. 3). Neither the maximal velocity of shortening ($+dL/dt$) nor that of relengthening ($-dL/dt$) was significantly different between the BBDR and BBDP myocytes (Fig. 2).

Effect of ethanol on myocyte shortening (PS)

Representative traces depicting the typical effect of ethanol ($240 \text{ mg dl}^{-1}$) on cell shortening in myocytes from BBDR or BBDP groups is shown in Fig. 1A. At the end of a 5 min exposure to this concentration of ethanol,
the PS was depressed by 35.3% and 25.4% in BBDR and BBDP myocytes, respectively. Acute ethanol exposure (80–640 mg dl⁻¹) caused a concentration-dependent inhibition of PS in both BBDR and BBDP myocytes (Fig. 1B). The maximal inhibition was 52.9% with a threshold between 80 and 120 mg dl⁻¹ in myocytes from the BBDR group. However, acute ethanol exposure produced less of an inhibition in cells from the BBDP group with a maximal inhibition of 28.4%. The threshold of inhibition observed in the BBDP group was between 80 and 120 mg dl⁻¹. The inhibitory effect on cell shortening was maximal within 4 min of exposure and was completely reversed upon washout.

Effect of ethanol on maximal velocity (+dL/dt) and duration (TPS and TR₉₀) of shortening and relengthening

As mentioned above, the baseline ±dL/dt was not significantly affected by chronic genetic diabetes. However, both +dL/dt and −dL/dt were depressed by acute ethanol exposure (80–640 mg dl⁻¹) in a concentration-dependent manner in myocytes from the BBDR and BBDP groups (Fig. 2). The degree of ethanol-induced inhibition of ±dL/dt was somewhat similar in myocytes from the two groups, with the exception that the threshold of inhibition for +dL/dt was higher (between 120 and 240 mg dl⁻¹) in

![Figure 2](image)

**Figure 2**

Effect of acute ethanol exposure (80–640 mg dl⁻¹) on maximal velocities of shortening (A: +dL/dt) and relengthening (B: −dL/dt) in myocytes from both BBDR and BBDP rat hearts. Means ± s.e.m., *P < 0.05 vs. 0 mg dl⁻¹.

![Figure 3](image)

**Figure 3**

Effect of acute ethanol exposure (80–640 mg dl⁻¹) on duration of shortening (A: TPS) and relengthening (B: TR₉₀) in myocytes from both BBDR and BBDP rat hearts. Means ± s.e.m., #P < 0.05 vs. BBDR group.
the BBDR group. The baseline TPS and TR90 were both significantly prolonged by genetic diabetes, consistent with our earlier report (Ren & Bode, 2000). However, neither TPS nor TR90 was significantly affected by acute ethanol exposure (Fig. 3).

DISCUSSION

The major finding of the present study is that the acute ethanol exposure-induced cardiac contractile depression in ventricular myocytes may be attenuated by genetically predisposed diabetes. The BB Wistar type 1 diabetic model is characterized by hyperglycaemia, reduced body weight gain and increased cardiac, hepatic and renal size, consistent with our previous finding (Ren & Bode, 2000). Acute ethanol exposure led to a concentration-dependent depression of myocyte peak shortening amplitude (PS) and maximal velocity of shortening and relengthening (±dL/dt) in myocytes isolated from both BBDR and BBDP groups. However, the ethanol-induced inhibition on PS but not ±dL/dt was significantly attenuated in myocytes from the BBDP diabetic group. The duration of shortening (TPS) and relengthening (TR90) was not affected by acute ethanol exposure in either the BBDR or BBDP group. These results confirmed our earlier observations (Ren & Davidoff, 1997; Ren & Bode, 2000) of prolonged duration of contraction and relaxation, characteristic of diabetic cardiomyopathy in genetically predisposed diabetic myocytes, and further indicated that diabetic myocytes may be less sensitive to the ethanol-induced cardiac depression.

Ethanol depresses cardiac contractility at both tissue and cellular levels (Danziger et al. 1991; Ren & Brown, 1999). Although several mechanisms have been postulated for alcoholic cardiomyopathy following chronic alcohol intake, including derangement of lipid metabolism (Baraona & Lieber, 1998), the detrimental effects of ethanol on cardiac contractile function may be simply due to its ability to interrupt one or more of the steps in cardiac excitation–contraction coupling such as membrane ion channels and pumps, SR Ca2+ release/resequestration, and contractile protein expression (Danziger et al. 1991; Thomas et al. 1994). Paradoxically, several of these excitation–contraction coupling components (e.g. myofilament Ca2+ responsiveness and membrane Ca2+ channels) may also be impaired by diabetes (Fein & Sonnenblick, 1994; Ren & Bode, 2000). It is therefore possible that reduced ethanol-induced cardiac depression in the BBDP myocytes, seen in the current study, may result from a ‘masking’ or ‘shadowing’ effect due to diabetes. For example, the voltage-dependent Ca2+ current may be inhibited by both diabetes (Wang et al. 1995) and ethanol (Thomas et al. 1994).

Epidemiological evidence has long suggested that there is significant interaction between alcohol intake and diabetes (Holbrook et al. 1990; Ajani et al. 2000; Kao et al. 2001). Historically, alcohol intake by diabetic patients has been controversial. Ethanol has been shown to exert beneficial effects on insulin resistance, high density lipoprotein (HDL) cholesterol, platelet aggregation, and fibrinolysis (Bell, 1996; Ajani et al. 2000), indicating that light to moderate use of ethanol should not be discouraged in diabetic patients. This notion seems to be consistent with the findings from the current study in that ethanol-induced cardiac depression was not exaggerated by the diabetic state. However, caution should be taken regarding the correct interpretation of the results from this current study. First, alcoholic cardiomyopathy is a chronic process whereas the current study only examined alcohol-induced cardiac depression after a few minutes of exposure. However, one extrapolation that could be made from the acute study to true alcoholic cardiomyopathy is that alcohol appears to depress the cardiac contractile function less in genetic diabetes, as opposed to being directly ‘beneficial’. As stated earlier, alcohol and diabetes may have similar cellular targets in cardiac myocytes so that further insult of alcohol on the ‘already compromised’ myocytes due to diabetes may not be obvious. Second, acute ethanol exposure-induced cardiac contractile defects may not possess the components of long-term alcohol intake-induced alteration in protein synthesis and lipid peroxidation (Preedy & Richardson, 1994). Further study is warranted to evaluate the myocyte mechanics in rats with concurrent diabetes and chronic alcoholic intake.

Contrary to the present observations, earlier reports from our group failed to detect a difference in ethanol-induced negative inotropic response between normal and streptozotocin-induced diabetic myocardium (Brown et al. 1993, 1996a). There are at least two plausible explanations for these discrepancies. First, Dr Brown used isolated left-ventricular papillary muscles whereas the current investigation employed ventricular myocytes devoid of any connective tissue, nerve terminals or other non-myocyte tissues. Secondly, Dr Brown used a chemically induced diabetic model whereas the current study was done in a genetically predisposed diabetic model. Another point worthy of mention is that the rats were studied at 12 months of age, which should have allowed the progression of diabetes into the advanced stage. In an earlier study by our group, the negative inotropic effect of ethanol was attenuated in older rats compared to younger ones (Brown et al. 1996b), suggesting that the age of the experimental animals may potentially affect the outcome of the study (even with the age-matched BBDR and BBDP rats used in the current study). A similar study is needed in rats at a younger age.

In conclusion, the present study revealed, for the first time, that ventricular myocytes from rats with genetically predisposed diabetes are less sensitive to acute ethanol exposure-induced cardiac contractile depression. These data should help our understanding of the inter-relationship between alcohol intake and diabetic heart dysfunction. Future investigation should be focused on the cellular and molecular mechanisms of action involved in the cardiomyopathies under both chronic alcoholism and diabetes.


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