ELSEVIER

Alcohol 46 (2012) 89–99

ALCOHOL

Ethanol causes endoplasmic reticulum stress and impairment of insulin secretion in pancreatic β-cells

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Received 31 January 2010; received in revised form 8 November 2010; accepted 29 April 2011

Abstract

Chronic ethanol consumption increases the risk of type 2 diabetes mellitus, and ethanol has been reported to cause insulin resistance and, inconsistently, to reduce insulin secretion. The mechanism(s) underlying the reduction of insulin secretion by ethanol is not known. We used β-cell lines and isolated murine islets to determine the effect of ethanol on insulin content and secretion at low- and high-glucose concentrations, in the presence of KCl, diazoxide, tolbutamide, and regulators of cyclic AMP and protein kinase C (PKC). We also determined the gene expression of insulin; pancreas duodenum homeobox 1; and endoplasmic reticulum (ER) stress markers, such as Chop, ERp57, glucose-regulated protein 78/binding immunoglobulin protein, and inositol 1,4,5-triphosphate receptors. Ethanol reduced insulin secretion by interfering with muscarinic signaling and PKC activation but not the K-ATP channels. In addition, ethanol reduced insulin content and caused ER stress. The deleterious effects of ethanol on β-cells were prevented by 4-methyl pyrazole, an inhibitor of alcohol dehydrogenase, suggesting that ethanol metabolism is required for these effects.

Keywords: Islets; Insulin secretion; Endoplasmic reticulum stress; Inositol trisphosphate receptor; Insulin genes; Beta-cell death

Introduction

Chronic, but not moderate, ethanol consumption increases the risk of type 2 diabetes mellitus (DM2) and this has been attributed to increased adiposity and insulin resistance (Carlsson et al., 2000; Holbrook et al., 1990; Sakuta et al., 2005; Wei et al., 2000). Several studies have shown that ethanol causes insulin resistance in the liver and skeletal muscle by interfering with insulin signaling (Onishi et al., 2003; Sasaki and Wands, 1994; Wan et al., 2005; Zhao et al., 2009). It is now well accepted that failure of β-cells to compensate for insulin resistance is a prerequisite for DM2 development, and chronic ethanol feeding in rodents has been reported to cause pancreatic β-cell apoptosis (Dembele et al., 2009; Lee et al., 2010) and to decrease β-cell mass (Koko et al., 1995; Russell et al., 1989; Zhao et al., 2009). Similar to brain and liver cells, chronic ethanol consumption might result in β-cell apoptosis because of mitochondrial dysfunction and oxidative stress caused by acetaldehyde, the highly reactive metabolite of ethanol, which forms adducts with cellular macromolecules (Adachi et al., 2004; Chu et al., 2007). Although it is expected that ethanol damages β-cells to explain the association of alcoholism with DM2, it is still controversial whether ethanol inhibits insulin secretion by β-cells, as both inhibition and enhancement of insulin secretion by ethanol have been reported (Hafko et al., 2009; Huang and Sjoholm, 2008; Koko et al., 1995; Metz et al., 1969; Nealon et al., 1988; Patel and Singh, 1979; Russell et al., 1989; Shin et al., 2002; Singh et al., 1980; Tiengo et al., 1981). Furthermore, little is known about mechanisms of ethanol's effects in β-cells. We have recently reported that ethanol causes insulinoma cell apoptosis through mitochondrial dysfunction as manifested by increased reactive oxygen species (ROS) production, reduction in ATP synthesis, and increased uncoupling protein 2 (Dembele et al., 2009; Lee et al., 2010). In the present study, we provide potential mechanisms explaining inhibition of insulin secretion by ethanol.

Material and methods

Roswell Park Memorial Institute (RPMI) 1640 medium (American Type Culture Collection [ATCC] no. 30-2001) was purchased from the ATCC (Manassas, VA). INS-1E cells were kindly provided by Dr. Michael Wheeler, University of

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0741-8329/$ - see front matter
doi: 10.1016/j.alcohol.2011.04.001

Toronto (Canada) with permission from Professor Claes Wollheim, University of Geneva (Switzerland). Fetal bovine serum, penicillin, streptomycin, dNTP Mix (dATP, dCTP, dGTP, dTTP), oligonucleotide primers, and Moloney murine leukemia virus reverse transcriptase were purchased from Invitrogen (Burlington, ON, Canada). Collagenase type V was from Roche Diagnostic (Mississauga, ON, Canada). EnzyChrom ethanol assay kit was from Bioassay Systems (Hayward, CA). 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and all other chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada). Microplates and cell culture flasks were from Corning (Corning, NY). Ultrasensitive mouse and rat insulin ELISA kits were purchased from Crystal Chem, Downer Grove, Illinois.

Cell culture and ethanol treatment

INS-1E cells (passages 10–15) were grown in RPMI 1640 medium containing 11 mM glucose, 2.5 mM glutamine, 1.8 mM sodium bicarbonate, 50 μM β-mercaptoethanol, 1 mM sodium pyruvate, 10% fetal bovine serum, 100 IU/mL penicillin, and 100 IU/mL streptomycin (growth medium). Cells were cultured in T75 flasks and maintained at 37°C in a humidified atmosphere of 5% CO_2 and passaged every 4–6 days by mild trypsinization. Briefly, cells were incubated for 3 min with 3 mLof 0.25% trypsin to which 3 mL growth medium were added. Stock solutions of ethanol were prepared in 0.1 M phosphate-buffered saline (PBS; pH 7.2) before dilution with growth medium and cell treatment, resulting in final concentrations of 20 and 80 mM. Cells were treated with ethanol with or without 100 μM 4-methyl pyrazole for 24 h. Control treatment consisted of growth medium containing 11 mM glucose without ethanol or 4-methyl pyrazole. In parallel experiments, ethanol was kept in flasks without cells for 24 or 48 h to evaluate the rate of evaporation and compared with the concentration of ethanol in flasks containing cells. Culture media were collected every 24 h and ethanol concentration was measured using the EnzyChrom ethanol assay kit following manufacturer's instructions. The ethanol concentrations were based on previous studies by us (Dembele et al., 2009; Lee et al., 2010) and others (Eysseric et al., 1997; He et al., 2007; Hong-Brown et al., 2001). The lower concentration is just above the legal limit for driving in North America, Britain, and other countries, whereas the higher concentration is within the range reported in human alcoholics (Touquet et al., 2008; Urso et al., 1981; Zuba et al., 2002).

To determine insulin release, INS-1E cells were preincubated in Krebs Ringer buffer (KRB) containing 118.4 mM NaCl, 4.7 mM KCl, 1.9 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3 (equilibrated with 5% CO2–95% O2, pH 7.4), 0.2% bovine serum albumin, 10 mM HEPES, with 2.2 mM glucose, and then incubated in KRB with 2.2 or 16.7 mM glucose for 30 min.

Cell viability

Before seeding and treatment, cell viability was checked by the trypan blue dye exclusion test. After treatment, cell viability was assessed by the MTT assay, a mitochondrial function assay based on the ability of viable cells to reduce the redox indicator MTT to insoluble formazan crystals by mitochondrial dehydrogenase (Mosmann, 1983). Briefly, cells were cultured at 8×10^4 cells/tube in 1.5 mL tube and incubated with 1 mg/mL of MTT dissolved in 500 μL of growth medium at 37°C for 2 h. The tube was then centrifuged at 2,000×g for 2 min and the supernatant was discarded. The resulting formazan crystals were solubilized in 400 μL DMSO divided into two wells of 98-well plates and read at 570 nm on FluoStar Optima using FluoStar optima system.

Islet insulin secretion studies

Islets were isolated by collagenase digestion of the pancreas from 28–32 g male CD1 mice (Lacy and Kostianovsky, 1967) as previously reported (Hoa et al., 2004, 2007). The islets were allowed to recover for 24 h in RPMI 1640 medium, containing 11.0 mM glucose, 10% heat inactivated fetal calf serum, 100 IU/mL penicillin, and 0.1 mg/mL streptomycin (Hoa et al., 2004) and then subsequently incubated for 24–48 h in a medium containing ethanol with or without 100 μM 4-methyl pyrazole. Three islets of equivalent size were washed in PBS for MTT assay. Glucose-induced insulin secretion (GIIS) was determined after batch incubation of islets in KRB with 3.3, 10.0, or 16.7 mM glucose. Batches of three islets of equivalent size were preincubated for 30 min at 3.3 mM glucose, and then incubated for 60 min in KRB with 3.3 mM or 10.0–16.7 mM glucose. To study the effect of ethanol on K-ATP channels, and sulfonylurea or muscarinic receptors, islets were incubated in ethanol for 48 h, then washed and incubated for 60 min in KRB containing 3.3 or 16.7 mM glucose with or without 0.25 mM diazoxide, 25 mM KCl, 150 μM tolbutamide or 100 μM carbachol. To study ethanol effect on cyclic AMP and protein kinase C (PKC) signaling, islets were incubated with 1.5 μM forskolin, 100 μM isobutylmethylxanthine (IBMX), 2.5 μM 12-O-tetradecanoylphorbol-13-acetate (TPA), or 1 μM Go 6976 in the presence of glucose. Islet insulin content was determined after islets preincubated with 3.3 or 16.7 mM glucose were lysed in 200 μL of 70% ethanol plus HCl (1.5% vol/vol) followed by sonication for 15 s (Garcia-Ocana et al., 2000). Samples were subsequently incubated overnight and then centrifuged at 13,000 rpm, and the supernatant was used to determine insulin content.

Real time PCR

Real time PCR was performed as described (Lee et al., 2010; Nammi et al., 2007). Briefly, total RNA was extracted from 0.2×10^6 INS-1E cells and 100 isolated islets by the