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Ethanol suppresses carbamylcholine-induced intracellular calcium oscillation in mouse pancreatic acinar cells

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ABSTRACT

Oscillation of intracellular calcium levels is closely linked to initiating secretion of digestive enzymes from pancreatic acinar cells. Excessive alcohol consumption is known to relate to a variety of disorders in the digestive system, including the exocrine pancreas. In this study, we have investigated the role and mechanism of ethanol on carbamylcholine (CCh)-induced intracellular calcium oscillation in murine pancreatic acinar cells. Ethanol at concentrations of 30 and 100 mM reversibly suppressed CCh-induced Ca^{2+} oscillation in a dose-dependent manner. Pretreatment of ethanol has no effect on the store-operated calcium entry induced by 10 μM of CCh. Ethanol significantly reduced the initial calcium peak induced by low concentrations of CCh and therefore, the CCh-induced dose-response curve of the initial calcium peak was shifted to the right by ethanol pretreatment. Furthermore, ethanol significantly dose-dependently reduced inositol 1,4,5-trisphosphate-induced calcium release from the internal stores in permeabilized acinar cells. These results provide evidence that excessive alcohol intake could impair cytosolic calcium oscillation through inhibiting calcium release from intracellular stores in mouse pancreatic acinar cells.

Introduction

The gastrointestinal system, which includes the esophagus, stomach, bowel, and pancreas, is a primary target for alcohol-induced disease. Alcohol consumption induces motility disorders and mucosal lesions under acute conditions in the esophagus, stomach, and bowel (Siegmund & Singer, 2005; Stermer, 2002). In the pancreas, alcohol causes the alteration of exocrine secretion that is affected by the dosage and duration of alcohol exposure, the additional administration of food, or the secretory state of the gland (Clemens et al., 2014). Alcohol consumption in humans with a meal caused significantly less pancreatic enzyme secretion compared with a meal alone (Hajnal, Flores, Radley, & Valenzuela, 1990). The exposure to ethanol *in vitro* resulted in decreased cholecystokinin (CCK)-induced enzyme secretion in isolated rat pancreatic acinar cells (Nakamura et al., 1991; Tachibana et al., 1996). The non-oxidative metabolites of ethanol are known to induce pancreatic acinar cell injury, rather than ethanol itself (Criddle et al., 2004; Wu, Bhopale, Ansari, & Kaphalia, 2008). Furthermore, the ethanol metabolites could reduce carbachol-stimulated or CCK-stimulated apical exocytosis and redirect the exocytosis to the basolateral plasma membrane (Cosen-Binker, Lam, Binker, & Gaisano, 2007; Dolai et al., 2012).

Pancreatic acinar cells synthesize and secrete a variety of digestive enzymes, and this function is tightly regulated by intracellular repetitive calcium transit, termed calcium oscillation (Petersen, 1982; Williams, 1980; Williams, Groblewski, Ohnishi, & Yule, 1997). A physiological concentration of carbamylcholine (CCh) could generate calcium oscillation that is known to be initiated by inositol 1,4,5-trisphospate-dependent calcium release from the intracellular calcium stores and be sustained by calcium entry from the extracellular medium (Petersen et al., 1991; Yule & Gallacher, 1988; Yule, Stuenkel, & Williams, 1996). The loaded calcium is rapidly redistributed to the internal stores or extracellular space through activation of sarco/endoplasmic reticulum calcium ATPase (SERCA), or plasma membrane calcium ATPase (PMCA),

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respectively. In this study, we aimed to investigate the role and underlying mechanism of ethanol on physiological concentrations of CCh-induced calcium oscillation in dispersed murine pancreatic acinar cells.

Materials and methods

Animals and reagents

Adult male Balb/c mice were used for this study. Mice were humanely handled and housed under specific pathogen-free conditions in clean polypropylene cages. Type II collagenase was purchased from Roche Diagnostics GmbH (Mannheim, Germany), fura-2/AM and magfura-2/AM were obtained from Thermo Fisher Scientific (Waltham, MA, USA), and inositol 1,4,5-trisphosphate ($InsP_3$) was obtained from Enzo Life Sciences (Farmingdale, NY, USA). All other materials were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of pancreatic acinar cells

Pancreatic acinar cells (8–12 cells per experiment) were freshly isolated by collagenase digestion as described previously (Park, Betzenhauser, Won, Chen, & Yule, 2008). Briefly, the pancreas was removed from mice after CO_2 asphyxiation and cervical dislocation. The dissected tissue was enzymatically digested for 30 min with type II collagenase in HEPES-buffered physiological saline containing 0.01% trypsin inhibitor (soybean) and 0.1% bovine serum albumin, followed by mechanical dissociation of the cells by gentle agitation. Cells were then filtered through 100-μm nylon mesh and centrifuged at 75× *g* with 1% bovine serum albumin. After isolation, pancreatic acinar cells were resuspended in HEPES-buffered physiological saline containing 137-mM NaCl, 4.7-mM KCl, 0.56-mM $MgCl_2$, 1-mM Na_2HPO_4, 10-mM HEPES, 1.28-mM $CaCl_2$, and 5.5-mM glucose (pH 7.4 adjusted with NaOH), until ready for use. To examine in a Ca^{2+}-free condition, HEPES-buffered physiological saline containing no added Ca^{2+} was supplemented with 5-mM EGTA. All experimental procedures were carried out in accordance with the NIH guidelines for the care and use of laboratory animals.

Measurements of cytosolic Ca^{2+} in intact cells

The isolated pancreatic acinar cells were loaded with 5-μM Fura-2/AM for 40 min at room temperature for measurements of intracellular Ca^{2+}. Fura-2/AM-loaded cells were mounted on a cover-glass at the bottom of perfusion chambers. Cells were continuously superfused with HEPES-buffered physiological saline using an electronically controlled perfusion system (Warner Instrument, Hamden, CT, USA). The fluorescence ratio of 340/380 nm was measured using Till-Photonics imaging system (Pleasanton, CA, USA). Fluorescence emission at 505 nm was detected using a cooled charged-coupled device, the Cool-SNAP HQ_2 camera (Photometrics, Tucson, AZ, USA) attached to an inverted microscope. All drugs were dissolved in HEPES-buffered physiological saline and were continuously perfused to the cells in the perfusion chamber at a flow rate of 1 mL/min using an electronic controlled perfusion system (Waner Instrument, Hamden, CT, USA).

Measurements of luminal Ca^{2+} in the endoplasmic reticulum in permeabilized cells

The isolated acinar cells were loaded with 5-μM magfura-2/AM for 40 min at room temperature and then attached on Cell-Tak- (BD Biosciences, San Jose, CA, USA) coated coverslips at the bottom of perfusion chambers. Cells were permeabilized by perfusion with 250-μM β-escin for 1–2 min in intracellular medium (ICM) containing 125-mM KCl, 19-mM NaCl, 10-mM HEPES, and 1-mM EGTA (pH 7.3) as described previously (Park, Betzenhauser, Zhang, & Yule, 2012). To remove the cytosolic dye, the permeabilized cells were washed in ICM without β-escin for 15 min. Intracellular Ca^{2+} stores were subsequently loaded with Ca^{2+} by activation of SERCA. To

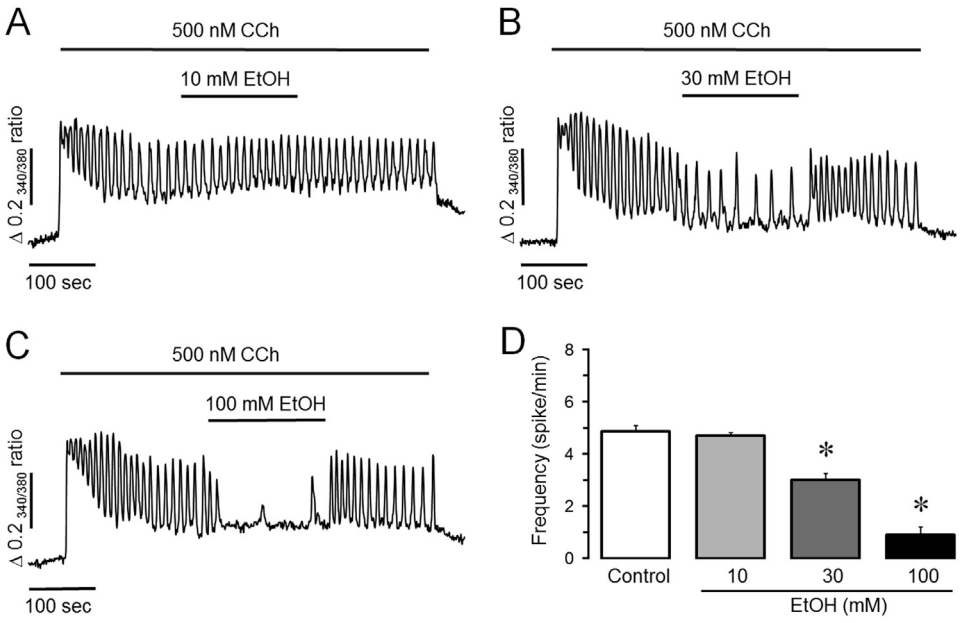

Fig. 1. Dose-dependent effect of ethanol on intracellular Ca^{2+} oscillation in intact pancreatic acinar cells. Effects of 10- (A), 30- (B), and 100-mM (C) ethanol on CCh (500 nM)-induced Ca^{2+} oscillation. (D) Effects of ethanol on frequency of Ca^{2+} spike. The various concentrations of ethanol were perfused during 200 s after steady-state Ca^{2+} oscillation induced by 500-nM CCh in HEPES buffer containing normal extracellular Ca^{2+}. Representative traces show the changes of 340/380 ratio. Data were obtained from 8, 6, 7, and 6 separate experiments for control, 10, 30, and 100 mM of ethanol-treated groups (58–78 cells). Asterisks indicate that the value is significantly different from the corresponding value of control ($p < 0.05$).