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Original Article

Hydrogen peroxide attenuates refilling of intracellular calcium store in mouse pancreatic acinar cells

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ABSTRACT Intracellular calcium (Ca^{2+}) oscillation is an initial event in digestive enzyme secretion of pancreatic acinar cells. Reactive oxygen species are known to be associated with a variety of oxidative stress-induced cellular disorders including pancreatitis. In this study, we investigated the effect of hydrogen peroxide (H_2O_2) on intracellular Ca^{2+} accumulation in mouse pancreatic acinar cells. Perfusion of H_2O_2 at 300 μM resulted in additional elevation of intracellular Ca^{2+} levels and termination of oscillatory Ca^{2+} signals induced by carbamylcholine (CCh) in the presence of normal extracellular Ca^{2+}. Antioxidants, catalase or DTT, completely prevented H_2O_2-induced additional Ca^{2+} increase and termination of Ca^{2+} oscillation. In Ca^{2+}-free medium, H_2O_2 still enhanced CCh-induced intracellular Ca^{2+} levels and thapsigargin (TG) mimicked H_2O_2-induced cytosolic Ca^{2+} increase. Furthermore, H_2O_2-induced elevation of intracellular Ca^{2+} levels was abolished under sarco/endoplasmic reticulum Ca^{2+} ATPase-inactivated condition by TG pretreatment with CCh. H_2O_2 at 300 μM failed to affect store-operated Ca^{2+} entry or Ca^{2+} extrusion through plasma membrane. Additionally, ruthenium red, a mitochondrial Ca^{2+} uniporter blocker, failed to attenuate H_2O_2-induced intracellular Ca^{2+} elevation. These results provide evidence that excessive generation of H_2O_2 in pathological conditions could accumulate intracellular Ca^{2+} by attenuating refilling of internal Ca^{2+} stores rather than by inhibiting Ca^{2+} extrusion to extracellular fluid or enhancing Ca^{2+} mobilization from extracellular medium in mouse pancreatic acinar cells.

INTRODUCTION

Reactive oxygen species (ROS) are formed as a result of partial reduction of oxygen during aerobic respiration [1]. They cause oxidative damage to various biological molecules including DNA, lipids, and proteins, thereby disrupting normal cellular function [2-4]. Under physiological conditions, ROS are controlled by intracellular free radical scavengers and antioxidant enzymes to protect cells from injuries [5]. However, imbalance between ROS generating and scavenging systems can lead to oxidative stress which can morphologically and functionally damage cells [6]. It is well-known that hydrogen peroxide (H_2O_2), one type of ROS, can disrupt normal functions in various cell types [2,3]. It is correlated with overloaded intracellular Ca^{2+} [7-9]. However, the mechanism of H_2O_2-induced Ca^{2+} accumulation has known to be complicated due to cell-to-cell difference in expression and participation of Ca^{2+} modulating transporters. It has been reported that H_2O_2 can enhance Ca^{2+} release from intracellular store [10-12], stimulate Ca^{2+} entry from extracellular medium [13-16], and attenuate Ca^{2+} extrusion by plasma membrane Ca^{2+} ATPase (PMCA) or sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) inactivation [17,18] in various cell types.

Author contributions: M.N.Y. performed Ca^{2+} measurement and analyzed the data. D.K.K. and S.H.K. conceived the idea and disigned the study. H.S.P. supervised and coordinated the study and reviewed the manuscript.

Pancreatic acinar cells synthesize and secrete a variety of digestive enzyme, tightly regulated by intracellular repetitive Ca^{2+} oscillation [19,20]. A physiological concentration of carbamylcholine (CCh) could generate Ca^{2+} oscillation known to be initiated by inositol 1,4,5-trisphospate receptors-mediated Ca^{2+} release from the intracellular store followed by activation of Ca^{2+} entry from extracellular medium [21,22]. The loaded Ca^{2+} is rapidly cleared to the internal store through SERCA or to the extracellular space through PMCA [23]. Overloaded Ca^{2+} can cause premature intracellular digestive enzyme activation and cellular injury, one of characteristics of pancreatitis [24,25].

Although the pathophysiology of pancreatitis remains unclear at the present time, it has been proposed that oxidative stress due to excess generation of ROS is involved in acute pancreatitis [26]. A prominent feature of acute pancreatitis is disruption of Ca^{2+} homeostasis within pancreatic acinar cells, and cytosolic Ca^{2+} accumulation has been shown to cause elevation of ROS in acinar cells that promote cell death [27]. Moreover, there are evidences showing that antioxidants can provide benefits to pancreatitis patients with pancreatic cell injury [28]. However, how ROS accumulates intracellular Ca^{2+} in pancreatic acinar cell is unclear at the present time. The objective of this study was to characterize the effect of H_2O_2 on CCh-induced intracellular Ca^{2+} signals and the underlying mechanism involved in Ca^{2+} accumulation in mouse pancreatic acinar cells. Here we report that H_2O_2 could accumulate intracellular Ca^{2+} by reducing refilling of intracellular Ca^{2+} stores, rather than by inhibiting Ca^{2+} extrusion to extracellular fluid or enhancing Ca^{2+} mobilization from extracellular medium in mouse pancreatic acinar cells.

METHODS

Animals

Male BALB/c mice at 8~10 weeks old were humanely handled and housed under specific pathogen-free conditions in clean polypropylene cages. They were maintained in air conditioned room at 20~22°C with a constant photoperiod of 12 hours light/dark cycle. Mice were provided free access to pallet diet and drinking water *ad libitum*. All animal experiments were performed in accordance with the Guideline for the Care and Use of Laboratory Animal provided by NIH. All experiments adhered to Konyang University policies regarding the care and use of animals.

Materials

Type II collagenase was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Fura-2/acetoxymethyl ester (fura-2/AM) was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Thapsigargin (TG) was purchased from Tocris (Avonmouth, BS, UK). All other materials were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of pancreatic acinar cells

Small clusters of pancreatic acinar cells (10~15 cells per experiment) were freshly isolated using collagenase digestion method as described previously [29,30]. Briefly, the pancreas was removed from mice after CO_2 asphyxiation and cervical dislocation. The dissected tissue was enzymatically digested with type II collagenase in HEPES-buffered physiological saline containing 0.01% trypsin inhibitor (soybean) and 0.1% bovine serum albumin (BSA) for 30 minutes followed by mechanical dissociation of cells by gentle agitation. Cells were then filtered through 100 μm nylon mesh and centrifuged at 75 g with 1% BSA. After isolation, 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 use. For Ca^{2+}-free condition, HEPES-buffered physiological saline without adding Ca^{2+} was supplemented with 5 mM ethylene glycol-bis (2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA).

Intracellular Ca^{2+} measurements

To measure intracellular Ca^{2+}, the isolated acinar cells were loaded with 5 μM Fura-2/AM and incubated at room temperature in dark condition for 40 minutes. Fura-2/AM loaded cells were mounted onto a cover-glass at the bottom of perfusion chambers. Cells were continuously perfused with HEPES-buffered physiological saline using an electronically controlled perfusion system (Warner Instrument, Hamden, CT, USA). Cells were excited alternately with light at 340 nm and 380 nm using a Polychrome V monochrometer (TILL Photonics, Pleasanton, CA, USA). Fluorescence emission at 505 nm was detected with a CoolSNAP HQ_2 camera (Photometrics, Tucson, AZ, USA) attached to an inverted microscope. The fluorescence ratio of 340/380 was measured using Till-Photonics imaging system. Stimuli were dissolved in HEPES-buffered physiological saline and used to continuously perfuse cells in the perfusion chamber at a flow rate of 1 ml/minute using an electronic controlled perfusion system (Waner Instrument, Hamden, CT, USA).

Data analysis

Values are expressed as mean±SEM. Student t test were used for data analysis. Differences were considered as statistically significant when the p value was less than 0.05. Ca^{2+} entry rates and extrusion rates were estimated by fitting the increasing and decreasing fluorescence to a single exponential function using Origin program.

RESULTS

Effects of hydrogen peroxide (H_2O_2) on CCh-induced intracellular Ca^{2+} oscillation

First, the effects of H_2O_2 on intracellular Ca^{2+} oscillation were performed in pancreatic acinar cells. Intracellular Ca^{2+} oscillation was evoked by 500 nM of CCh perfusion in the presence of extracellular Ca^{2+} at 1.28 mM in intact cells. As shown in Fig. 1A, CCh at 500 nM generated repetitive and sustained Ca^{2+} oscillation. After the steady state, perfusion of H_2O_2 at 300 μM resulted in additional elevation of intracellular Ca^{2+} levels and termination of Ca^{2+} oscillation in 97±4% cells (n=7, 98 cells). These effects were irreversible even when H_2O_2 was washed out. Since, in preliminary study, only small proportion of cells were response to H_2O_2 at 100 μM (34±3%, n=5, 73 cells), we used H_2O_2 at a concentration of 300 μM in the following studies. Additionally, pretreatment of antioxidants such as catalase at 30

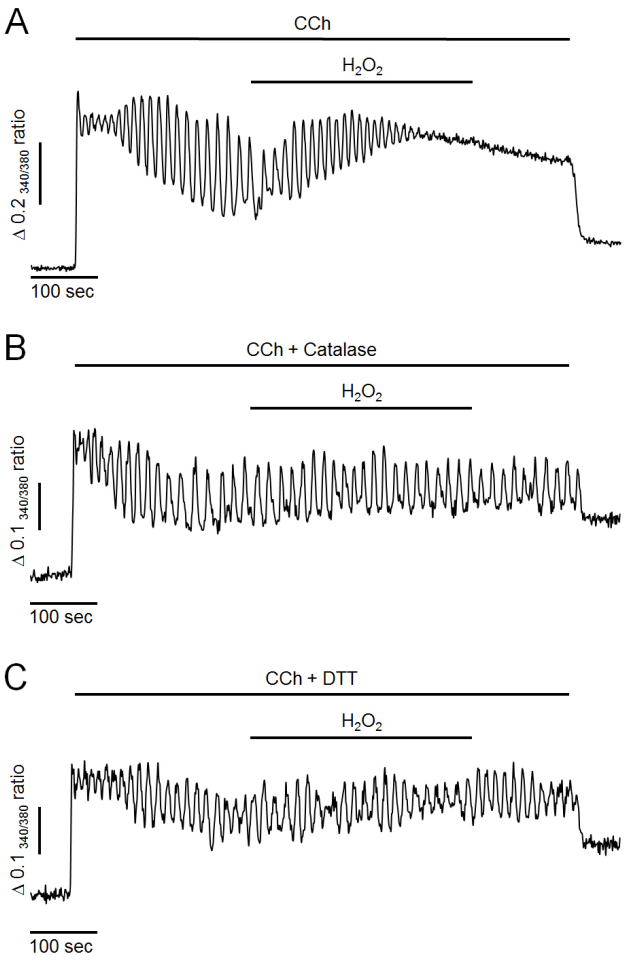

Fig. 1. Effects of hydrogen peroxide (H_2O_2) and antioxidants on CCh-induced intracellular Ca^{2+} oscillation in intact pancreatic acinar cells. (A) Representative trace showing the effect of H_2O_2 on CCh-induced Ca^{2+} oscillation. (B, C) Representative traces showing the effects of antioxidants (30 μg/ml of catalase and 2 mM of DTT) on H_2O_2-induced intracellular Ca^{2+} changes. Oscillatory Ca^{2+} signals were induced by perfusion with 500 nM of CCh in HEPES buffer containing normal extracellular Ca^{2+}. H_2O_2 at 300 μM was perfused for 5 minutes. All data were obtained from at least five separate experiments (71~98 cells) and expressed as changes of 340/380 ratio. The perfusion of H_2O_2 resulted in an elevation of intracellular Ca^{2+} concentration and a termination of Ca^{2+} oscillation. Antioxidants completely prevent H_2O_2-induced Ca^{2+} accumulation.

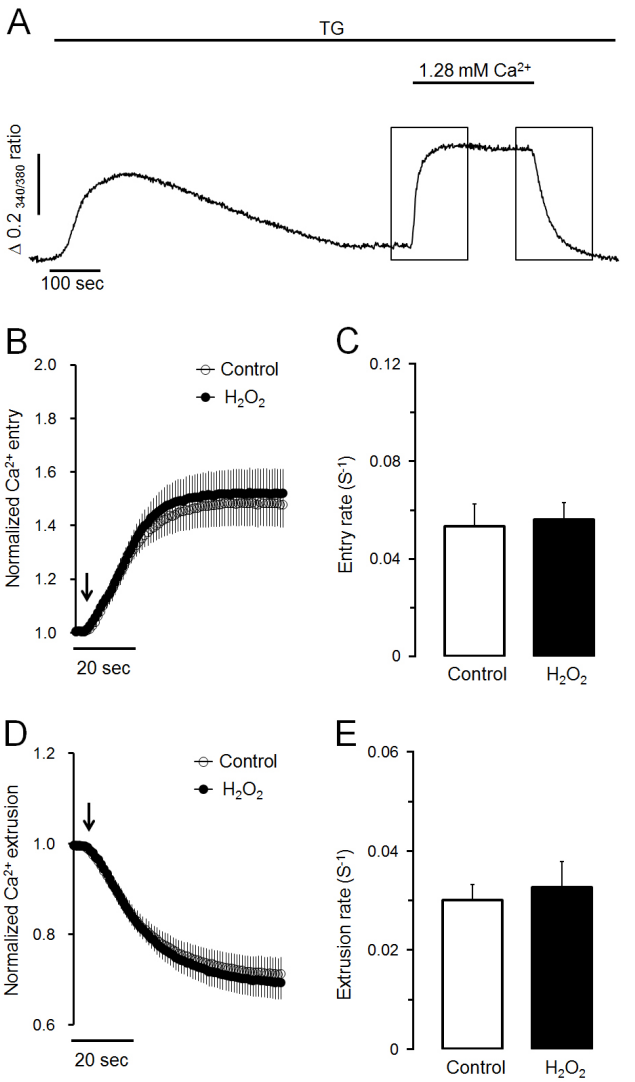

Fig. 2. H_2O_2 does not affect Ca^{2+} entry or Ca^{2+} extrusion in TG-treated pancreatic acinar cells. (A) Representative trace showing the effect of SERCA inactivation using TG on Ca^{2+} entry from extracellular medium and Ca^{2+} extrusion to extracellular medium. To deplete intracellular Ca^{2+} stores, TG at 1 μM was treated in Ca^{2+}-free medium. After depletion of intracellular Ca^{2+} stores, 1.28 mM of Ca^{2+} was added and removed to activate Ca^{2+} entry and Ca^{2+} extrusion, respectively. (B, C) Effects of H_2O_2 on normalized Ca^{2+} entry and Ca^{2+} entry rate in TG-treated pancreatic acinar cells. Values are expressed as means±SEM obtained from six separate experiments (76 cells). (D, E) Effects of H_2O_2 on normalized Ca^{2+} extrusion and Ca^{2+} extrusion rate in TG-treated cells. H_2O_2 at 300 μM did not modify Ca^{2+} entry or Ca^{2+} extrusion through plasma membrane in TG-treated pancreatic acinar cells.

μg/ml or 1,4-dithiothreitol (DTT) at 2 mM with CCh completely prevented the effects of H_2O_2 (i.e., the additional elevation of intracellular Ca^{2+} levels and the termination of Ca^{2+} oscillation) (Fig. 1B, C). These results suggest that H_2O_2 could accumulate intracellular Ca^{2+} and disrupt normal oscillatory Ca^{2+} signals in mouse pancreatic acinar cells.

H_2O_2 does not affect Ca^{2+} entry or Ca^{2+} extrusion through plasma membrane

Next, we determined whether H_2O_2-induced Ca^{2+} accumulation was caused by facilitating Ca^{2+} entry from extracellular medium or reducing Ca^{2+} extrusion to extracellular medium through plasma membrane. As shown in Fig. 2A, Ca^{2+} store was initially depleted with 1 μM of TG in Ca^{2+}-free medium. Store-operated Ca^{2+} entry was then stimulated by adding extracellular Ca^{2+} at 1.28 mM. Ca^{2+} extrusion through plasma membrane was then stimulated by changing to Ca^{2+}-free medium in intact cells. In the control experiment, the adding of extracellular Ca^{2+} remarkably stimulated Ca^{2+} entry from extracellular fluid with a Ca^{2+} entry rate of 0.053±0.009 S^{-1}. H_2O_2-induced Ca^{2+} entry rate was 0.056±0.007 S^{-1}, which was not significantly different from the control value (Fig. 2B, C). In H_2O_2-treated cells, the removing of extracellular Ca^{2+} clearly extruded intracellular Ca^{2+} to external space (Fig. 2A). The Ca^{2+} extrusion rate was 0.033±0.005 S^{-1}, which was not significantly different from its control value at 0.030±0.003 S^{-1} (Fig. 2D, E). Thus, neither Ca^{2+} entry from extracellular medium nor Ca^{2+} extrusion to extracellular medium was modified by H_2O_2 treatment. Therefore, H_2O_2-induced Ca^{2+} accumulation was not due to facilitating Ca^{2+} entry from extracellular medium or reducing Ca^{2+} extrusion to extracellular medium through plasma membrane in pancreatic acinar cells.

TG mimics H_2O_2-induced Ca^{2+} responses and pretreatment of TG completely abolishes H_2O_2-induced Ca^{2+} responses in Ca^{2+}-free medium

Next, we evaluated whether H_2O_2 could elevate intracellular Ca^{2+} levels in Ca^{2+}-free medium because H_2O_2 did not facilitate Ca^{2+} entry or reduce Ca^{2+} extrusion through plasma membrane. As shown in Fig. 3A, in Ca^{2+}-free medium, 500 nM of CCh resulted in Ca^{2+} oscillation in the initial state, indicating that CCh initially mobilized Ca^{2+} from intracellular stores. However, oscillatory signals were ceased and returned to baseline levels after 300~500 sec of CCh perfusion in Ca^{2+}-free medium due to discontinued Ca^{2+} supply from extracellular fluid. After 200 sec of CCh perfusion, treatment with 300 μM H_2O_2 still resulted in an additional elevation of intracellular Ca^{2+} levels even when extracellular Ca^{2+} was eliminated (Fig. 3B). Furthermore, additional elevation of intracellular Ca^{2+} concentration was mimicked by TG treatment in Ca^{2+}-free medium (Fig. 3C). However, the H_2O_2-induced additional increase of Ca^{2+} was completely abolished under SERCA-inactivated condition by TG pretreatment with CCh. Since, in this condition, intracellular TG-sensitive Ca^{2+} stores were already depleted, TG-insensitive other Ca^{2+} pools may not participate on H_2O_2-induced additional elevation of intracellular Ca^{2+} concentration. These results suggested that H_2O_2 could accumulate intracellular Ca^{2+} through inhibiting Ca^{2+} refilling to intracellular store by inactivation of SERCA, similar to the effect of TG.

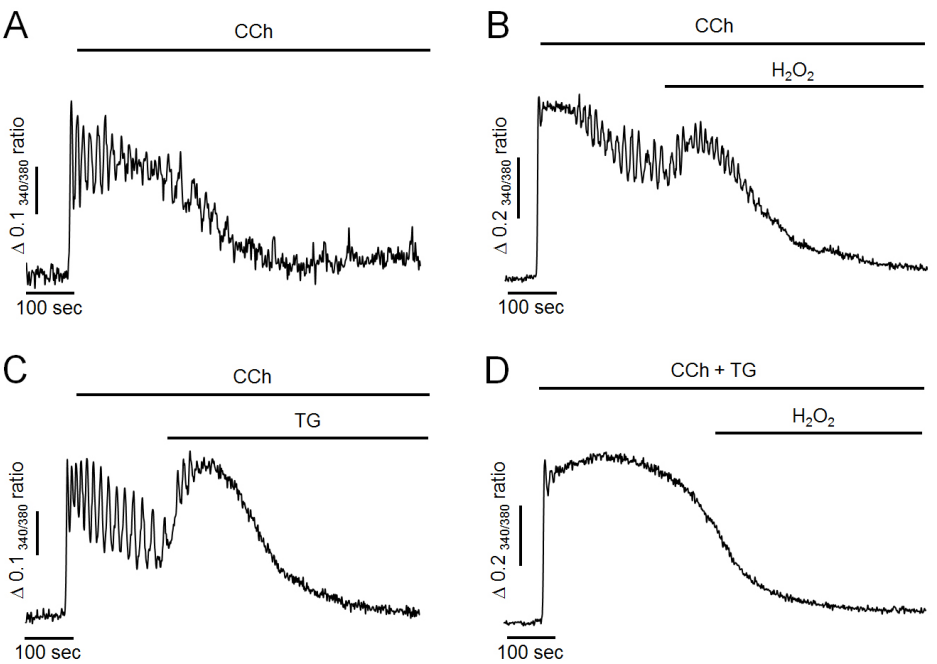

Fig. 3. Effects of H_2O_2 and TG on CCh-induced intracellular Ca^{2+} response in Ca^{2+}-free medium. (A) Representative trace showing CCh-induced intracellular Ca^{2+} response in Ca^{2+}-free medium. (B) H_2O_2 -induced additional elevation of intracellular Ca^{2+} levels. (C) TG mimicked the additional elevation of intracellular Ca^{2+} levels. (D) Pretreatment of TG with CCh completely abolished H_2O_2-induced additional elevation of intracellular Ca^{2+} levels. All data were obtained from at least five separate experiments (74~103 cells). Perfusion of H_2O_2 at 300 μM resulted in additional elevation of intracellular Ca^{2+} levels, which was mimicked by 1 μM of TG perfusion in Ca^{2+}-free medium. H_2O_2-induced additional increase of Ca^{2+} was completely abolished by inactivation of SERCA with TG pretreatment.

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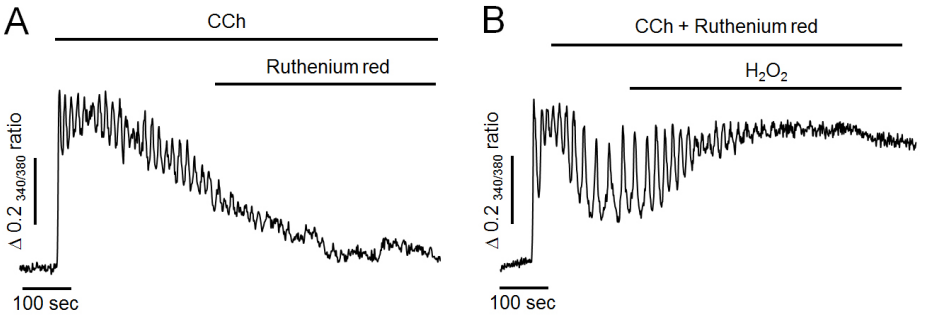

Fig. 4. Effect of ruthenium red on H_2O_2-induced intracellular Ca^{2+} response in Ca^{2+}-free medium. (A) Representative trace showing the effect of ruthenium red on CCh-induced intracellular Ca^{2+} response in Ca^{2+}-free medium. (B) Pretreatment of ruthenium red with CCh failed to attenuate H_2O_2-induced additional elevation of intracellular Ca^{2+} levels. All data were obtained from six and seven separate experiments (70 and 81 cells). Perfusion of ruthenium red at 50 μM, a mitochondrial Ca^{2+} uniporter inhibitor, did not mimic H_2O_2-induced additional elevation of intracellular Ca^{2+} levels. After pretreatment of ruthenium red with CCh, H_2O_2 still elevated intracellular Ca^{2+} concentration.

Ruthenium red does not attenuate H_2O_2-induced Ca^{2+} responses in Ca^{2+}-free medium

To further determine whether H_2O_2 could reduce mitochondrial Ca^{2+} buffering effect, ruthenium red at 50 μM, a mitochondrial Ca^{2+} uniporter blocker, was used in the following experiment. As shown in Fig. 4A, ruthenium red has no any effect to compare the control experiment (Fig. 3A), and failed to mimic H_2O_2-induced additional elevation of intracellular Ca^{2+} levels in Ca^{2+}-free medium. Thus, it is unlikely that mitochondria remarkable participate on Ca^{2+} elimination after CCh stimulation. Moreover, H_2O_2 still elevated intracellular Ca^{2+} levels even when mitochondrial uniporter was blocked by pretreatment of ruthenium red with CCh (Fig. 4B). This result indicated that Ca^{2+} accumulation induced by H_2O_2 might not be by reducing Ca^{2+}-buffering capacity of mitochondria in pancreatic acinar cells.

DISCUSSION

The present study clearly provided evidence that H_2O_2, a reactive oxygen species, could accumulate cytosolic Ca^{2+} through attenuating refilling of intracellular Ca^{2+} store in mouse pancreatic acinar cells. Cytosolic free Ca^{2+} plays a pivotal role in the stimulus-secretion coupling process in pancreatic acinar cells [19,20]. Ca^{2+} can be mobilized to elicit physiological responses from both the external fluid and the internal stores such as endoplasmic reticulum and acidic store. Acetylcholine and cholecystokinin (CCK), the major agonists in pancreatic exocrine gland, are known to generate repetitive and transient oscillatory Ca^{2+} signals [21,22]. The balance between Ca^{2+} mobilization and Ca^{2+} elimination is important to generate Ca^{2+} oscillation in exocrine cells. These processes are regulated by the action of a variety of channels, pumps, and exchangers for Ca^{2+} localized both in the plasma membrane and the ER membrane [19,20]. Since the accumulation of intracellular Ca^{2+} causes cellular damage associated with acute and chronic pancreatitis [24,25], basal intracellular Ca^{2+} concentrations have to be finely regulated to low resting values under normal condition.

Although oxidant-induced intracellular Ca^{2+} overload has been revealed in various cell types, the underlying mechanisms of Ca^{2+} mobilization and elimination are complicated [10-18]. It has been known that the involvement of oxidants in Ca^{2+} homeostasis is mediated by the modification of disulfide bonds between cysteine residues of Ca^{2+} regulating proteins including SERCA, PMCA and Na^+/Ca^{2+} exchanger (NCX) [31,32]. These molecules have different isoforms with different expression characteristics and regulation properties, thus giving versatility of Ca^{2+} signaling [33]. The present study was designed to investigate the exact mechanism of how H_2O_2 could cause intracellular Ca^{2+} accumulation in pancreatic acinar cells. When acinar cells were exposed to 300 μM of H_2O_2 in normal buffer, there was additional elevation of cytosolic Ca^{2+} and termination of oscillatory Ca^{2+} signals. These effects of H_2O_2 on Ca^{2+} signals were completely prevented by pretreatment with catalase (an enzyme that can degrade hydrogen peroxide) and DTT (a sulfhydryl reducing agent). Although cytosolic H_2O_2 concentrations produced by oxidative stress in pancreatic acinar cells is not known, only small proportion of cells (34%) were response to 100 μM of H_2O_2 and most cells (97%) were response to 300 μM of H_2O_2 in the present study. In general, H_2O_2 at concentrations from 10 μM to 1 mM caused intracellular Ca^{2+} accumulation in various cell types [10-17]. These results suggest that excess generation of oxidants in pathologic conditions could disturb Ca^{2+} homeostasis mediated by sulfhydryl group oxidation in pancreatic acinar cells.

Next, we investigated whether H_2O_2 actually induced Ca^{2+} entry from extracellular fluid through plasma membrane. In ventricular myocyte, ROS can enhance Ca^{2+} entry through modulating the function of voltage-gated L-type Ca^{2+} channels in plasma membrane [34]. It has been reported that ROS play physiological roles in platelet aggregation by activating SOC-mediated Ca^{2+} entry in human platelets [35]. Transient receptor

potential (TRP) channels, such as TRPC3, TRPM2, TRPM7, and TRPPA1 are also known to sensitive to ROS [36]. They participate in neurodegeneration process of neuronal cells. However, H_2O_2-induced Ca^{2+} accumulation still occurred in Ca^{2+}-free medium in this study after extracellular Ca^{2+} sources were eliminated. Furthermore, H_2O_2 failed to attenuate SOC-mediated Ca^{2+} entry by adding extracellular Ca^{2+} at 1.28 mM after ER Ca^{2+} stores were depleted in Ca^{2+}-free medium by pretreatment of TG. In pancreatic acinar cells, evidence of the existence or the role of voltage-gated Ca^{2+} channels or NCX has not been presented. The role of TRP channels and their sensitivities to H_2O_2 have also not been fully elucidated at the present time. Our results suggested that Ca^{2+} entry channels in plasma membrane might not be the primary targets of H_2O_2-induced Ca^{2+} accumulation in mouse pancreatic acinar cells.

In this study, H_2O_2-induced additional elevation of intracellular Ca^{2+} concentration was mimicked by TG treatment in Ca^{2+}-free medium. Moreover, H_2O_2-induced additional increase of Ca^{2+} was completely abolished in SERCA-inactivated condition by TG pretreatment with CCh. These results strongly suggest that H_2O_2 could accumulate intracellular Ca^{2+} through inhibiting refilling to intracellular Ca^{2+} store, similar to the effect of TG by inactivating SERCA. Since SERCA contains 20~28 cysteine residues, its activity can be effectively modulated by oxidants. It has been reported that ROS could attenuate the activity of this pump by modifying sulfhydryl groups [37]. Distinct SERCA isoforms are known to show different susceptibilities to ROS due to different location of cysteine residues [38]. In rat pancreatic acinar cell, there was no expression of SERCA1 mRNA and SERCA2 mRNA expression was down-regulated in acute pancreatitis [39]. The different sensitivity to H_2O_2 between SERCA subtypes is not known at the present time. Thus further studies are needed to elucidate the mechanism of H_2O_2 on calcium accumulation and cell damage. PMCA also could contribute to ROS-induced cytosolic Ca^{2+} accumulation because this pump has abundant cysteine residues [37]. In this study, 300 μM of H_2O_2 failed to attenuate Ca^{2+} extrusion through plasma membrane in TG-treated experiment. However 10 folds higher concentration of H_2O_2 partially inhibited Ca^{2+} extrusion to extracellular fluid under similar conditions (data not shown). These findings strongly suggest that the primary target for H_2O_2-induced Ca^{2+} accumulation might be SERCA rather than PMCA in mouse pancreatic acinar cells.

The overloaded Ca^{2+} also could be eliminated by buffering action of mitochondria. CCK can evoke oscillatory Ca^{2+} signals and substantial mitochondrial Ca^{2+} uptake in pancreatic acinar cells [40,41]. H_2O_2 can cause mitochondrial Ca^{2+} release abolished by pretreatment of FCCP or CCCP, a mitochondrial uncoupler [40,41]. However, in another study, mitochondrial Ca^{2+} uptake did not occur in unstimulated resting cells [18]. In addition, H_2O_2-induced mitochondrial Ca^{2+} uptake was very slow at low capacity even cells were stimulated by CCK [18]. In the present study, ruthenium red alone has no effect on CCh-induced Ca^{2+} response in Ca^{2+} free medium, and H_2O_2 still elevated intracellular Ca^{2+} levels even when mitochondrial uniporter was blocked by pretreatment of ruthenium red with CCh. Thus, it is unlikely that mitochondria are the major source of H_2O_2-induced elevation of cytosolic Ca^{2+}.

Based on the above results, we conclude that the primary target molecule for excessively generated H_2O_2 in pathological conditions is likely to be the sulfhydryl group of SERCA. We also conclude that H_2O_2 can accumulate intracellular Ca^{2+} by attenuating the refilling of intracellular Ca^{2+} stores through ER membrane rather than by Ca^{2+} entry or Ca^{2+} extrusion through plasma membrane in mouse pancreatic acinar cells.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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