

Original Article

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

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ABSTRACT Intracellular calcium (Ca²⁺) 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 (H₂O₂) on intracellular Ca²⁺ accumulation in mouse pancreatic acinar cells. Perfusion of H₂O₂ at 300 μM resulted in additional elevation of intracellular Ca²⁺ levels and termination of oscillatory Ca²⁺ signals induced by carbamylcholine (CCh) in the presence of normal extracellular Ca²⁺. Antioxidants, catalase or DTT, completely prevented H₂O₂-induced additional Ca²⁺ increase and termination of Ca²⁺ oscillation. In Ca²⁺-free medium, H₂O₂ still enhanced CCh-induced intracellular Ca²⁺ levels and thapsigargin (TG) mimicked H₂O₂-induced cytosolic Ca²⁺ increase. Furthermore, H₂O₂-induced elevation of intracellular Ca²⁺ levels was abolished under sarco/endoplasmic reticulum Ca²⁺ ATPase-inactivated condition by TG pretreatment with CCh. H₂O₂ at 300 μM failed to affect store-operated Ca²⁺ entry or Ca²⁺ extrusion through plasma membrane. Additionally, ruthenium red, a mitochondrial Ca²⁺ uniporter blocker, failed to attenuate H₂O₂-induced intracellular Ca²⁺ elevation. These results provide evidence that excessive generation of H₂O₂ in pathological conditions could accumulate intracellular Ca²⁺ by attenuating refilling of internal Ca²⁺ stores rather than by inhibiting Ca²⁺ extrusion to extracellular fluid or enhancing Ca²⁺ 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 (H₂O₂), one type of ROS, can disrupt normal functions in various cell types [2,3]. It is correlated with overloaded intracellular Ca²⁺ [7-9]. However, the mechanism of H₂O₂-induced Ca²⁺ accumulation has known to be complicated due to cell-to-cell difference in expression and participation of Ca²⁺ modulating transporters. It has been reported that H₂O₂ can enhance Ca²⁺ release from intracellular store [10-12], stimulate Ca²⁺ entry from extracellular medium [13-16], and attenuate Ca²⁺ extrusion by plasma membrane Ca²⁺ ATPase (PMCA) or sarco/endoplasmic reticulum Ca²⁺ ATPase (SERCA) inactivation [17,18] in various cell types.



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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-trisphosphate 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 monochromator (TILL Photonics, Pleasanton, CA, USA). Fluorescence emission at 505 nm was detected with a CoolSNAP HQ₂ 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 \pm 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 (H₂O₂) on CCh-induced intracellular Ca²⁺ oscillation

First, the effects of H₂O₂ on intracellular Ca²⁺ oscillation were performed in pancreatic acinar cells. Intracellular Ca²⁺ oscillation was evoked by 500 nM of CCh perfusion in the presence of extracellular Ca²⁺ at 1.28 mM in intact cells. As shown in Fig. 1A, CCh at 500 nM generated repetitive and sustained Ca²⁺ oscillation. After the steady state, perfusion of H₂O₂ at 300 μM

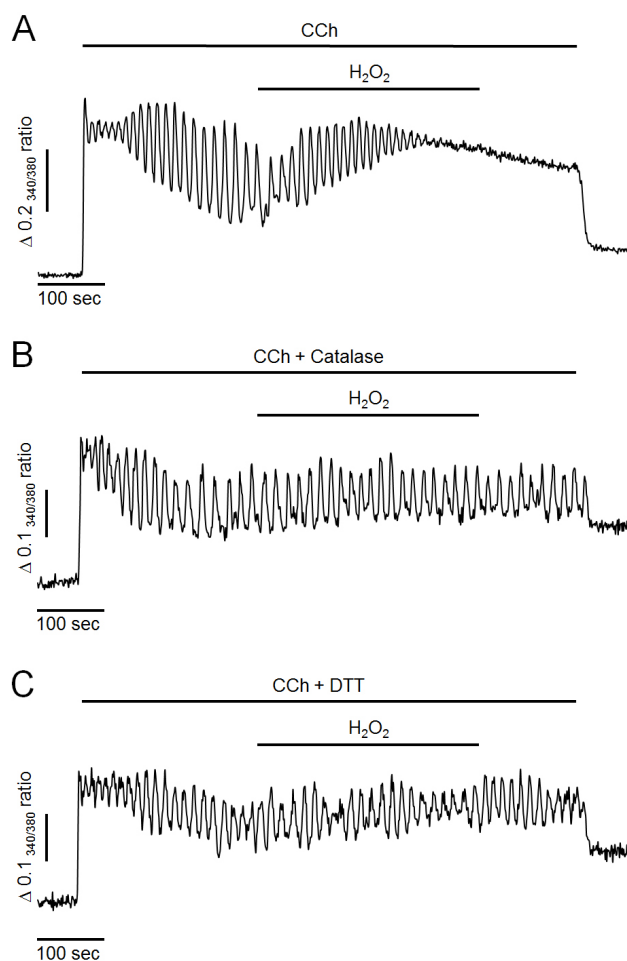


Fig. 1. Effects of hydrogen peroxide (H₂O₂) and antioxidants on CCh-induced intracellular Ca²⁺ oscillation in intact pancreatic acinar cells. (A) Representative trace showing the effect of H₂O₂ on CCh-induced Ca²⁺ oscillation. (B, C) Representative traces showing the effects of antioxidants (30 μg/ml of catalase and 2 mM of DTT) on H₂O₂-induced intracellular Ca²⁺ changes. Oscillatory Ca²⁺ signals were induced by perfusion with 500 nM of CCh in HEPES buffer containing normal extracellular Ca²⁺. H₂O₂ 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 H₂O₂ resulted in an elevation of intracellular Ca²⁺ concentration and a termination of Ca²⁺ oscillation. Antioxidants completely prevent H₂O₂-induced Ca²⁺ accumulation.

resulted in additional elevation of intracellular Ca²⁺ levels and termination of Ca²⁺ oscillation in 97±4% cells (n=7, 98 cells). These effects were irreversible even when H₂O₂ was washed out. Since, in preliminary study, only small proportion of cells were response to H₂O₂ at 100 μM (34±3%, n=5, 73 cells), we used H₂O₂ at a concentration of 300 μM in the following studies. Additionally, pretreatment of antioxidants such as catalase at 30

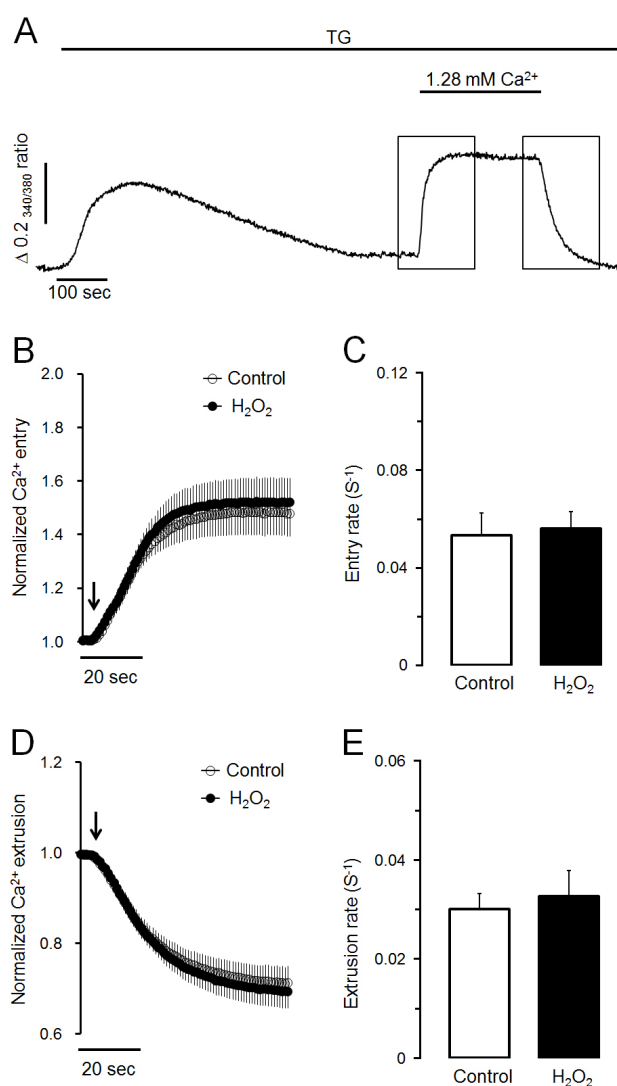


Fig. 2. H₂O₂ does not affect Ca²⁺ entry or Ca²⁺ extrusion in TG-treated pancreatic acinar cells. (A) Representative trace showing the effect of SERCA inactivation using TG on Ca²⁺ entry from extracellular medium and Ca²⁺ extrusion to extracellular medium. To deplete intracellular Ca²⁺ stores, TG at 1 μM was treated in Ca²⁺-free medium. After depletion of intracellular Ca²⁺ stores, 1.28 mM of Ca²⁺ was added and removed to activate Ca²⁺ entry and Ca²⁺ extrusion, respectively. (B, C) Effects of H₂O₂ on normalized Ca²⁺ entry and Ca²⁺ 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 H₂O₂ on normalized Ca²⁺ extrusion and Ca²⁺ extrusion rate in TG-treated cells. H₂O₂ at 300 μM did not modify Ca²⁺ entry or Ca²⁺ extrusion through plasma membrane in TG-treated pancreatic acinar cells.

$\mu\text{g/ml}$ 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 $0.053 \pm 0.009 \text{ S}^{-1}$. H_2O_2 -induced Ca^{2+} entry rate was $0.056 \pm 0.007 \text{ S}^{-1}$, 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 $0.033 \pm 0.005 \text{ S}^{-1}$, which was not significantly different from its control value at $0.030 \pm 0.003 \text{ S}^{-1}$ (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.

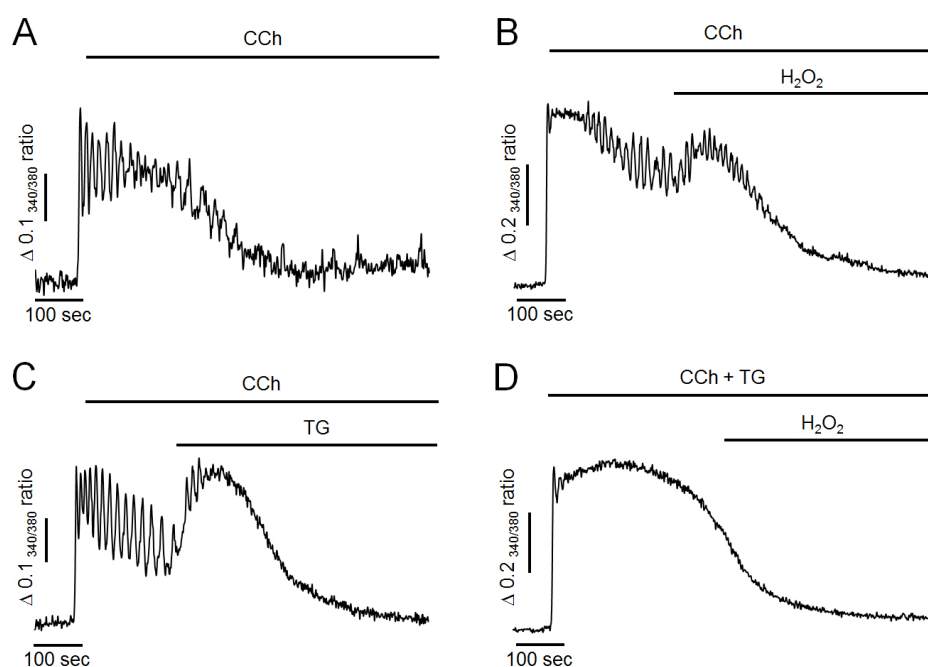


Fig. 3. Effects of H_2O_2 and TG on CCh-induced intracellular Ca^{2+} response in Ca^{2+} -free medium. (A) Representative trace showing CCh-induced intracellular Ca^{2+} response in Ca^{2+} -free medium. (B) H_2O_2 -induced additional elevation of intracellular Ca^{2+} levels. (C) TG mimicked the additional 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.

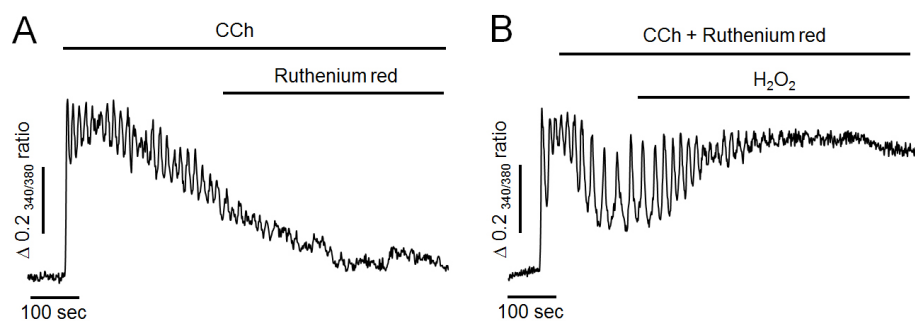


Fig. 4. Effect of ruthenium red on H₂O₂-induced intracellular Ca²⁺ response in Ca²⁺-free medium. (A) Representative trace showing the effect of ruthenium red on CCh-induced intracellular Ca²⁺ response in Ca²⁺-free medium. (B) Pretreatment of ruthenium red with CCh failed to attenuate H₂O₂-induced additional elevation of intracellular Ca²⁺ levels. All data were obtained from six and seven separate experiments (70 and 81 cells). Perfusion of ruthenium red at 50 μM, a mitochondrial Ca²⁺ uniporter inhibitor, did not mimic H₂O₂-induced additional elevation of intracellular Ca²⁺ levels. After pretreatment of ruthenium red with CCh, H₂O₂ still elevated intracellular Ca²⁺ concentration.

Ruthenium red does not attenuate H₂O₂-induced Ca²⁺ responses in Ca²⁺-free medium

To further determine whether H₂O₂ could reduce mitochondrial Ca²⁺ buffering effect, ruthenium red at 50 μM, a mitochondrial Ca²⁺ 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 H₂O₂-induced additional elevation of intracellular Ca²⁺ levels in Ca²⁺-free medium. Thus, it is unlikely that mitochondria remarkable participate on Ca²⁺ elimination after CCh stimulation. Moreover, H₂O₂ still elevated intracellular Ca²⁺ levels even when mitochondrial uniporter was blocked by pretreatment of ruthenium red with CCh (Fig. 4B). This result indicated that Ca²⁺ accumulation induced by H₂O₂ might not be by reducing Ca²⁺-buffering capacity of mitochondria in pancreatic acinar cells.

DISCUSSION

The present study clearly provided evidence that H₂O₂, a reactive oxygen species, could accumulate cytosolic Ca²⁺ through attenuating refilling of intracellular Ca²⁺ store in mouse pancreatic acinar cells. Cytosolic free Ca²⁺ plays a pivotal role in the stimulus-secretion coupling process in pancreatic acinar cells [19,20]. Ca²⁺ 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 Ca²⁺ signals [21,22]. The balance between Ca²⁺ mobilization and Ca²⁺ elimination is important to generate Ca²⁺ oscillation in exocrine cells. These processes are regulated by the action of a variety of channels, pumps, and exchangers for Ca²⁺ localized both in the plasma membrane and the ER membrane [19,20]. Since the accumulation of intracellular Ca²⁺ causes cellular

damage associated with acute and chronic pancreatitis [24,25], basal intracellular Ca²⁺ concentrations have to be finely regulated to low resting values under normal condition.

Although oxidant-induced intracellular Ca²⁺ overload has been revealed in various cell types, the underlying mechanisms of Ca²⁺ mobilization and elimination are complicated [10-18]. It has been known that the involvement of oxidants in Ca²⁺ homeostasis is mediated by the modification of disulfide bonds between cysteine residues of Ca²⁺ regulating proteins including SERCA, PMCA and Na⁺/Ca²⁺ exchanger (NCX) [31,32]. These molecules have different isoforms with different expression characteristics and regulation properties, thus giving versatility of Ca²⁺ signaling [33]. The present study was designed to investigate the exact mechanism of how H₂O₂ could cause intracellular Ca²⁺ accumulation in pancreatic acinar cells. When acinar cells were exposed to 300 μM of H₂O₂ in normal buffer, there was additional elevation of cytosolic Ca²⁺ and termination of oscillatory Ca²⁺ signals. These effects of H₂O₂ on Ca²⁺ signals were completely prevented by pretreatment with catalase (an enzyme that can degrade hydrogen peroxide) and DTT (a sulfhydryl reducing agent). Although cytosolic H₂O₂ concentrations produced by oxidative stress in pancreatic acinar cells is not known, only small proportion of cells (34%) were response to 100 μM of H₂O₂ and most cells (97%) were response to 300 μM of H₂O₂ in the present study. In general, H₂O₂ at concentrations from 10 μM to 1 mM caused intracellular Ca²⁺ accumulation in various cell types [10-17]. These results suggest that excess generation of oxidants in pathologic conditions could disturb Ca²⁺ homeostasis mediated by sulfhydryl group oxidation in pancreatic acinar cells.

Next, we investigated whether H₂O₂ actually induced Ca²⁺ entry from extracellular fluid through plasma membrane. In ventricular myocyte, ROS can enhance Ca²⁺ entry through modulating the function of voltage-gated L-type Ca²⁺ channels in plasma membrane [34]. It has been reported that ROS play physiological roles in platelet aggregation by activating SOC-mediated Ca²⁺ 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, H₂O₂-induced Ca²⁺ accumulation still occurred in Ca²⁺-free medium in this study after extracellular Ca²⁺ sources were eliminated. Furthermore, H₂O₂ failed to attenuate SOC-mediated Ca²⁺ entry by adding extracellular Ca²⁺ at 1.28 mM after ER Ca²⁺ stores were depleted in Ca²⁺-free medium by pretreatment of TG. In pancreatic acinar cells, evidence of the existence or the role of voltage-gated Ca²⁺ channels or NCX has not been presented. The role of TRP channels and their sensitivities to H₂O₂ have also not been fully elucidated at the present time. Our results suggested that Ca²⁺ entry channels in plasma membrane might not be the primary targets of H₂O₂-induced Ca²⁺ accumulation in mouse pancreatic acinar cells.

In this study, H₂O₂-induced additional elevation of intracellular Ca²⁺ concentration was mimicked by TG treatment in Ca²⁺-free medium. Moreover, H₂O₂-induced additional increase of Ca²⁺ was completely abolished in SERCA-inactivated condition by TG pretreatment with CCh. These results strongly suggest that H₂O₂ could accumulate intracellular Ca²⁺ through inhibiting refilling to intracellular Ca²⁺ 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 H₂O₂ between SERCA subtypes is not known at the present time. Thus further studies are needed to elucidate the mechanism of H₂O₂ on calcium accumulation and cell damage. PMCA also could contribute to ROS-induced cytosolic Ca²⁺ accumulation because this pump has abundant cysteine residues [37]. In this study, 300 μM of H₂O₂ failed to attenuate Ca²⁺ extrusion through plasma membrane in TG-treated experiment. However 10 folds higher concentration of H₂O₂ partially inhibited Ca²⁺ extrusion to extracellular fluid under similar conditions (data not shown). These findings strongly suggest that the primary target for H₂O₂-induced Ca²⁺ accumulation might be SERCA rather than PMCA in mouse pancreatic acinar cells.

The overloaded Ca²⁺ also could be eliminated by buffering action of mitochondria. CCK can evoke oscillatory Ca²⁺ signals and substantial mitochondrial Ca²⁺ uptake in pancreatic acinar cells [40,41]. H₂O₂ can cause mitochondrial Ca²⁺ release abolished by pretreatment of FCCP or CCCP, a mitochondrial uncoupler [40,41]. However, in another study, mitochondrial Ca²⁺ uptake did not occur in unstimulated resting cells [18]. In addition, H₂O₂-induced mitochondrial Ca²⁺ 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 Ca²⁺ response in Ca²⁺ free medium, and H₂O₂ still elevated intracellular Ca²⁺ 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 H₂O₂-induced elevation of cytosolic Ca²⁺.

Based on the above results, we conclude that the primary target molecule for excessively generated H₂O₂ in pathological conditions is likely to be the sulfhydryl group of SERCA. We also conclude that H₂O₂ can accumulate intracellular Ca²⁺ by attenuating the refilling of intracellular Ca²⁺ stores through ER membrane rather than by Ca²⁺ entry or Ca²⁺ 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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