Acta Pharmacologica Sinica (2014) 35: 1514–1520
 1671-4083/14 $32.00
www.nature.com/aps

Original Article

Congo red modulates ACh-induced Ca^{2+} oscillations in single pancreatic acinar cells of mice

Ze-bing HUANG[1, 2, 4], Hai-yan WANG[3], Na-na SUN[3], Jing-ke WANG[3], Meng-qin ZHAO[3], Jian-xin SHEN[3], Ming GAO[2], Ronald P HAMMER Jr[4], Xue-gong FAN[1, *], Jie WU[1, 2, 3, 4, *]

[1]Department of Infectious Diseases, Xiangya Hospital, Central South University, and Key Laboratory of Viral Hepatitis, Changsha 410008, China; [2]Divisions of Neurology and Neurobiology, Barrow Neurological Institute, St Joseph's Hospital and Medical Center, Phoenix, AZ 8501, USA; [3]Department of Physiology, Shantou University of Medical College, Shantou 210854, China; [4]Departments of Basic Medical Sciences, Pharmacology and Psychiatry, University of Arizona College of Medicine, Phoenix, AZ 85004, USA

Aim: Congo red, a secondary diazo dye, is usually used as an indicator for the presence of amyloid fibrils. Recent studies show that congo red exerts neuroprotective effects in a variety of models of neurodegenerative diseases. However, its pharmacological profile remains unknown. In this study, we investigated the effects of congo red on ACh-induced Ca^{2+} oscillations in mouse pancreatic acinar cells *in vitro*.

Methods: Acutely dissociated pancreatic acinar cells of mice were prepared. A U-tube drug application system was used to deliver drugs into the bath. Intracellular Ca^{2+} oscillations were monitored by whole-cell recording of Ca^{2+}-activated Cl^- currents and by using confocal Ca^{2+} imaging. For intracellular drug application, the drug was added in pipette solution and diffused into cell after the whole-cell configuration was established.

Results: Bath application of ACh (10 nmol/L) induced typical Ca^{2+} oscillations in dissociated pancreatic acinar cells. Addition of congo red (1, 10, 100 μmol/L) dose-dependently enhanced Ach-induced Ca^{2+} oscillations, but congo red alone did not induce any detectable response. Furthermore, this enhancement depended on the concentrations of ACh: congo red markedly enhanced the Ca^{2+} oscillations induced by ACh (10–30 nmol/L), but did not alter the Ca^{2+} oscillations induced by ACh (100–10000 nmol/L). Congo red also enhanced the Ca^{2+} oscillations induced by bath application of IP_3 (30 μmol/L). Intracellular application of congo red failed to alter ACh-induced Ca^{2+} oscillations.

Conclusion: Congo red significantly modulates intracellular Ca^{2+} signaling in pancreatic acinar cells, and this pharmacological effect should be fully considered when developing congo red as a novel therapeutic drug.

Keywords: Congo red; pancreatic acinar cell; ACh; IP_3; Ca^{2+} oscillation; whole-cell recording; Ca^{2+} image

Acta Pharmacologica Sinica (2014) 35: 1514–1520; doi: 10.1038/aps.2014.94; published online 27 Oct 2014

Introduction

Congo red is a secondary diazo dye that is usually used as an indicator for the presence of amyloid fibrils and is one of the model compounds that has been extensively studied for its interaction with amyloid beta peptide (Aβ)[1–5]. In addition, it has been found that congo red can block Aβ-formed ion channels[6, 7]. Because congo red can stabilize Aβ monomers, markedly decrease the oligomer/monomer ratio, inhibit amyloid fibril formation, and reduce Aβ-amyloid neurotoxicity[8, 9], it has been thought to be a candidate for the development of an alternative therapeutic treatment of AD[5]. However, the effects of congo red on cellular biology are not well investigated, and whether congo red modulates intracellular Ca^{2+} signaling is unknown.

Pancreatic acinar cells play an important role in producing and secreting enzymes for the proper digestion of food. Elevated cytosolic free calcium concentration ($[Ca^{2+}]_i$) is the critical early signal that triggers the release of digestive enzymes from pancreatic acinar cells[10, 11]. Acetylcholine (ACh) and cholecystokinin (CCK) are two typical agonists that activate these intracellular Ca^{2+} oscillations, and such agonist-induced Ca^{2+} oscillations have been well studied as a classical cellular model of intracellular Ca^{2+} signaling[12]. The agonist (ACh or CCK) binds to its respective surface membrane receptor to activate second messenger (inositol 1,4,5-trisphosphate [IP_3])-mediated Ca^{2+} release from cytoplasmic stores, such

* To whom correspondence should be addressed.
E-mail jie.wu@dignityhealth.org
Received 2014-05-03 Accepted 2014-08-19

as the endoplasmic reticulum (ER)[13, 14], and to induce $[Ca^{2+}]_i$ elevation. Thereafter, Ca^{2+} ATPase pumps on the plasma membrane of the ER are activated to transport Ca^{2+} from the cytosol back to the ER. Meanwhile, the depletion of intracellular Ca^{2+} stores can activate store-operated or Ca^{2+} release-activated Ca^{2+} entry (CRAC)[15, 16] to refill Ca^{2+} pools from the extracellular environment. Together, these mechanisms maintain the agonist-induced Ca^{2+} oscillations in the presence of persistent extracellular agonists. This is an ideal cell model with which to study the effects of various new drugs on intracellular Ca^{2+} signaling.

In the present study, we evaluate the effect of congo red on agonist-induced Ca^{2+} oscillations in mouse pancreatic acinar cells using both patch-clamp whole-cell recording (measurement of Ca^{2+}-dependent Cl^- current) and Ca^{2+} imaging approaches. We found that congo red modulates ACh-induced Ca^{2+} oscillations and that this modulation depends on the concentrations of both congo red and ACh. Congo red itself cannot induce any detectable Ca^{2+} responses under patch-clamp whole-cell recording conditions, and congo red modulates Ca^{2+} oscillations from extracellular, but not intracellular, targets. Collectively, these data suggest that congo red can modulate intracellular Ca^{2+} signaling in pancreatic acinar cells and that this pharmacological effect should be fully considered when developing congo red as a novel therapeutic drug.

Materials and methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee at the Barrow Neurological Institute.

Acutely dissociated mouse pancreatic acinar cells

Single pancreatic acinar cells were dissociated as previously described[17–19]. Briefly, adult (4–6 months old) male CD1 mice were anesthetized with isoflurane, and then the pancreas was rapidly removed and injected with collagenase (200 U/mL) dissolved in standard extracellular solution(see below). The tissue was treated with collagenase (200 U/mL) for 20–25 min at 37 °C. Following collagenase digestion, the cell suspension was gently pipetted to further dissociate the cells and then washed three times using oxygenated standard external solution. Next, 150 μL of the suspension was transferred into 2 mL of standard extracellular solution in a 35-mm culture dish. The isolated cells were usually utilized within 3 h after dissociation for patch-clamp recording or Ca^{2+} imaging.

Patch-clamp, whole-cell recording

Ca^{2+}-activated Cl^- currents were recorded using conventional whole-cell recordings to monitor intracellular Ca^{2+} signal oscillations as previously reported[17–19]. When the recording pipette was filled with the K^+-containing pipette solution, the resistance was 3–4 MΩ. After the formation of a GΩ seal between the cell membrane and pipette, a whole-cell recording mode was achieved by further brief suction. For perforated-patch recordings, the recording electrodes were filled with amphotericin B (150–200 μmol/L) dissolved in pipette solution (see below). The holding potential (V_H) for patch recording was -30 mV, and the series-resistance was not compensated. Transmembrane currents were recorded with a patch-clamp amplifier (Axopatch 200B; Molecular Devices; Sunnyvale, CA USA).

Confocal imaging to measure Ca^{2+} oscillations

Single pancreatic cells were first incubated with the Ca^{2+} indicator, Fluo-4-AM (15 μmol/L) (Molecular Probes, Eugene, OR USA) for 15 min, followed by a 10 min incubation period to allow for de-esterification of the indicator. Confocal imaging was performed using an Olympus FluoView FV1000 microscope (Olympus Corporation) equipped with an argon laser (488 nm) and an UPLSAPO 40×, 0.95 NA objective. X–Y imaging was performed at a rate of 1.644 s per frame, while acquiring 400 frames in total with a resolution of 512×512. The fluorescent Fluo-4 signal was measured using ImageJ v.1.47 (available from the US National Institutes of Health, Bethesda, MD USA; http://imagej.nih.gov/ij/).

Solution and chemicals

The standard extracellular solution contained (in mmol/L) 140 NaCl, 1.0 $CaCl_2$, 4.7 KCl, 1.13 $MgCl_2$, 10 glucose, and 10 HEPES, adjusted to pH 7.2 with NaOH. The pipette solution contained (in mmol/L) 140 KCl, 1.13 $MgCl_2$, 5 Na_2ATP, 0.24 EGTA, 10 glucose and 10 HEPES, adjusted to pH 7.2. Congo red (Figure 1), ACh, amphotericin B and IP_3 were purchased from Sigma-Aldrich (St Louis, MO USA). 2-APB was purchased from Tocris Bioscience (Minneapolis, MN USA). Congo red (Supplemental Figure 1) was dissolved with distilled water into a 10 mmol/L stock solution and was diluted with external solution to the final concentrations just before use. During patch recording, the recorded cell was continuously perfused with a stream of standard extracellular solution. A U-tube drug application system was used to deliver drugs via the bath. For intracellular drug applications, the test drug was added to pipette solution, and the establishment of the whole-cell configuration allowed the drug to diffuse into the cell.

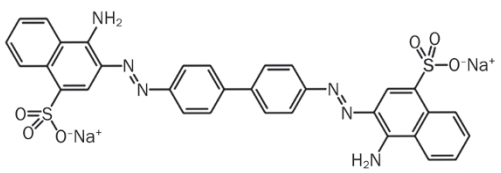

Figure 1. Chemical structure of congo red.

Statistical analysis

For patch clamp recordings, the data were analyzed by measured current area (Clampfit 9.2, pCLAMP; Molecular Devices). The net charge of the current was obtained by dividing the current area by the cell membrane capacitance (Cm)

over a certain time (usually for 3 min). Control values were measured from the baseline of ACh (or IP_3)-induced Ca^{2+} oscillations for approximately 3 min (normalized to 1) and were compared to the changes induced by congo red exposure. For Ca^{2+} imaging, the amplitude of the Ca^{2+} signal was calculated as: $\Delta R=\Delta F/F_0$, where F_0 refers to the background Fluo-4 signal. Student's *t*-test was used to analyze paired or unpaired data. A value of $P<0.05$ was considered statistically significant.

Results

Effects of congo red on ACh-induced Ca^{2+}oscillations

In the initial experiments, we tested the effects of congo red on ACh-induced Ca^{2+} oscillations. Bath application of 10 nmol/L ACh induced typical Ca^{2+} oscillations under patch-clamp whole-cell recordings (Figure 2A). Different concentrations of congo red were applied to the recorded cell in the presence of 10 nmol/L ACh, which potentiated ACh-induced current net charge in a concentration-dependent manner (Figure 2A–2D). These results suggest that congo red significantly enhances Ca^{2+} oscillations induced by 10 nmol/L ACh in a concentration-dependent manner.

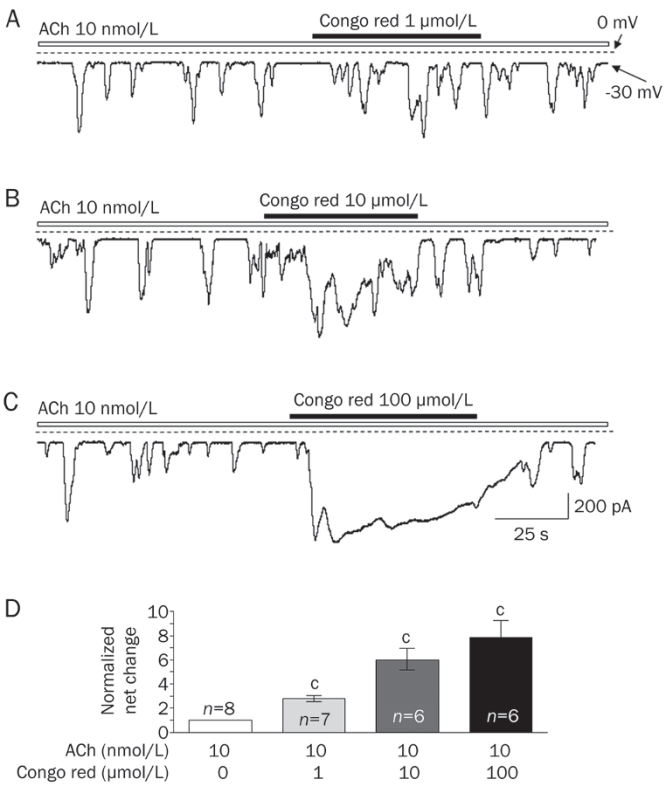

Figure 2. Congo red potentiates ACh-induced Ca^{2+} oscillations in a concentration-dependent manner. (A–C) Representative traces showing that in the presence of 10 nmol/L ACh, bath-applied congo red enhanced Ca^{2+} oscillations in a concentration-dependent manner. Traces (A–C) were recorded from the same cell. (D) The bar graph indicates potentiation of the net charge of the normalized current in the ACh-induced response by different concentrations of congo red. In this and the following figures, the numbers inside each column indicate the number of cells tested. The vertical bars represent the Mean±SEM. $^cP<0.01$.

Does congo red alone induce intracellular Ca^{2+} oscillations?

Next, we addressed this question. Congo red alone at various concentrations did not induce any detectable response under patch-clamp recording conditions (Figure 3A–3C). However, after congo red exposure, application of ACh (10 nmol/L) induced enhanced Ca^{2+} oscillation responses in a congo red concentration-dependent manner (Figure 3D). These results suggest that congo red does not directly promote intracellular Ca^{2+} release, but it does modulate ACh-induced Ca^{2+} oscillations.

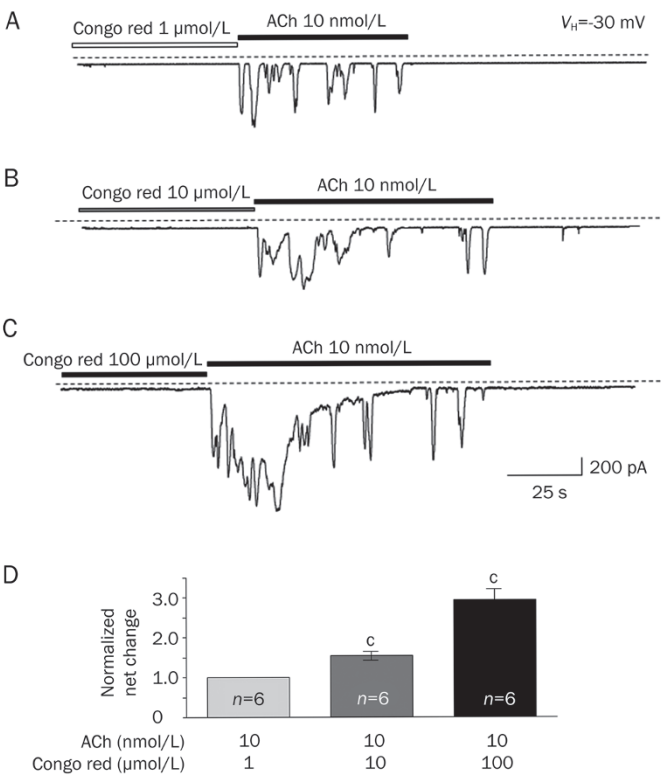

Figure 3. Congo red alone fails to induce a detectable current response under patch-clamp recording conditions. (A–C) Bath-applied congo red alone at different concentrations failed to induce any detectable current response. However, when ACh (10 nmol/L) was immediately bath-applied after congo red application, it induced enhanced Ca^{2+} oscillations in a concentration-dependent manner. (D) The bar graph summarizes the effects of ACh-induced current responses (normalized net charge) with different concentrations of congo red pretreatment. $^cP<0.01$. These results suggest that congo red itself is not able to induce Ca^{2+} oscillations, but it clearly potentiates ACh-induced Ca^{2+} oscillations.

Effects of congo red on different concentrations of ACh-induced Ca^{2+}oscillations

Next, we examined the effects of 10 μmol/L congo red on different concentrations of ACh-induced current responses. At low concentrations of ACh (5–30 nmol/L), congo red potentiated ACh-induced Ca^{2+} oscillations (Figure 4Aa). However, at higher concentrations of ACh (100 nmol/L) that usually

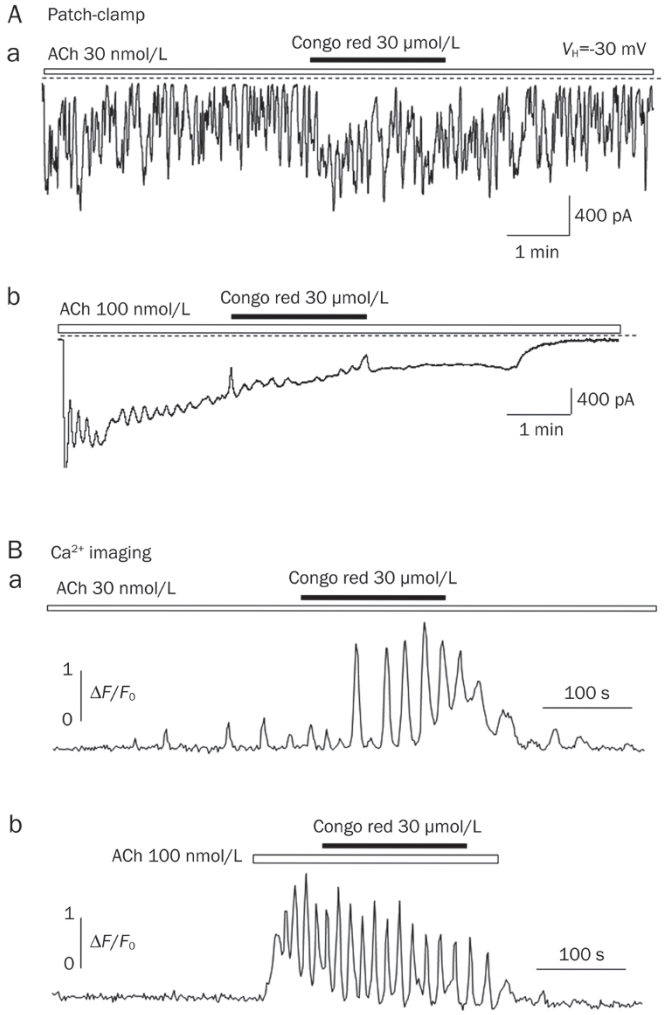

Figure 4. Effects of congo red on different concentrations of ACh-induced Ca^{2+} oscillations. (A) Representative traces of patch-clamp, whole-cell recordings, in which congo red (30 μmol/L) potentiated 30 nmol/L ACh-induced Ca^{2+} oscillations (Aa), but not 100 nmol/L ACh-induced Ca^{2+} responses (Ab). The trace shown represents the similar effects of congo red observed in the 5 other cells tested. (B) Representative traces of Ca^{2+} imaging, in which congo red (30 μmol/L potentiated 30 nmol/L ACh-induced Ca^{2+} oscillations (Ba), but not 100 nmol/L ACh-induced Ca^{2+} responses (Bb). A similar effect of congo red to that shown in Ba was observed in 66 cells (from 5 mice), and similar effects to those shown in Bb were observed in 44 cells (from 3 mice).

induced a plateau response with limited oscillations, congo red did not alter the ACh-induced response (Figure 4Ab). Similar effects of congo red on ACh-induced Ca^{2+} responses were also observed using Ca^{2+} imaging (Figure 4Ba, 4Bb), whereby 30 μmol/L congo red enhanced the 30 nmol/L ACh-induced Ca^{2+} response to 420.7%±4.5% (n=65 cells from 5 mice, P<0.01) of the control value, while 30 μmol/L congo red did not alter the 100 nmol/L ACh-induced Ca^{2+} response (95.1%±6.5%; n=44 cells from 3 mice, P>0.05). ACh concentration-dependent responses, suggesting that the 100 nmol/L ACh-induced Ca^{2+} response is not the maximal response (Supplemental Figure 1).

These results suggest that congo red modulation of ACh-induced Ca^{2+} oscillations also depends on ACh concentrations.

Effects of congo red on IP_3-induced Ca^{2+}oscillations

Data presented thus far clearly show that congo red enhances low concentration of ACh-induced Ca^{2+} oscillations. What is the mechanism of this modulation? The first possibility is that congo red may modulate muscarinic receptor function. To test this possibility, we examined the effects of congo red on intracellular IP_3-induced Ca^{2+} oscillations, because such Ca^{2+} oscillations bypass ACh receptors. IP_3 (30 μmol/L) infusion from the recording electrode into the recorded cell induced pulsatile Ca^{2+} oscillations (Figure 5Aa), and bath-applied congo red (10 μmol/L) dramatically enhanced these Ca^{2+} oscillations (Figure 5Ab). Similar results obtained from the 6 cells tested (Figure 5B) suggest that cell surface ACh receptors appear to be unnecessary for the mediation of the congo red-induced potentiation of Ca^{2+} oscillatory responses.

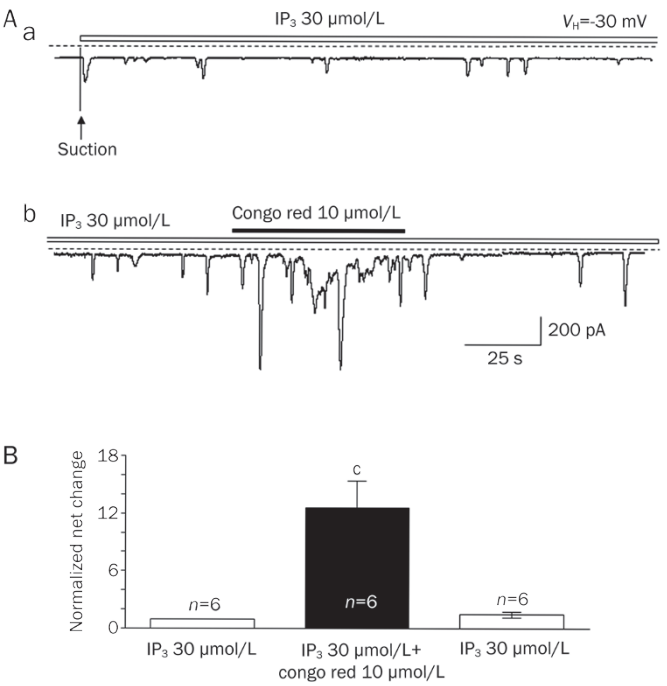

Figure 5. Effects of congo red on the Ca^{2+} oscillations induced by intracellular application of IP_3. (Aa) A typical trace showing that when the recording electrode contained 30 μmol/L IP_3, the formation of a whole-cell recording by suction of the recording electrode (indicated by an arrow) induced pulsatile Ca^{2+} oscillations. (Ab) Under conditions of intracellular IP_3-induced Ca^{2+} oscillations, bath-application of 10 μmol/L congo red enhanced the IP_3 response. (B) The bar graph summarizes the data regarding IP_3-induced Ca^{2+} oscillations before, during and after washout of congo red. ^bP<0.05, ^cP<0.01.

Effects of intracellular congo red on ACh-induced Ca^{2+}oscillations

To determine whether congo red can enhance agonist-induced Ca^{2+} oscillations through intracellular sites, we added 10

μmol/L congo red to the recording electrode and determined the effect of bath-application of ACh (10 nmol/L) after the establishment of a whole-cell recording configuration (infusion of congo red into the cytosol). For this experiment, we initially performed perforated patch recordings to examine the effect of bath-application of congo red on ACh-induced Ca^{2+} oscillations, and then we produced a brief suction to switch the recording mode to conventional whole-cell recording (Figure 6A). In the absence of congo red in the recording electrode, this switch slightly enhanced the ACh-induced oscillation response due to reduced access resistance (from perforated to whole-cell recording), as shown in the control recording in Figure 6A. Then, we compared the effects of this switch with the effects of congo red in the pipette solution (Figure 6Ba) and found there were no differences between the two conditions (Figure 6A and 6Ba, see the statistical comparison in Figure 6C), suggesting the congo red did not enhance Ca^{2+} oscillations when it was applied via the recording electrode. To confirm this, we applied the same concentration of congo red via bath application to the same recorded cell (in this cell, 10 μmol/L congo red was already infused via the recording electrode), and found a dramatic enhancement of Ca^{2+} oscillations (Figure 6Bb, C). These results suggest that congo red potentiates 10 nmol/L ACh-induced Ca^{2+} oscillations through extracellular, rather than intracellular, targets.

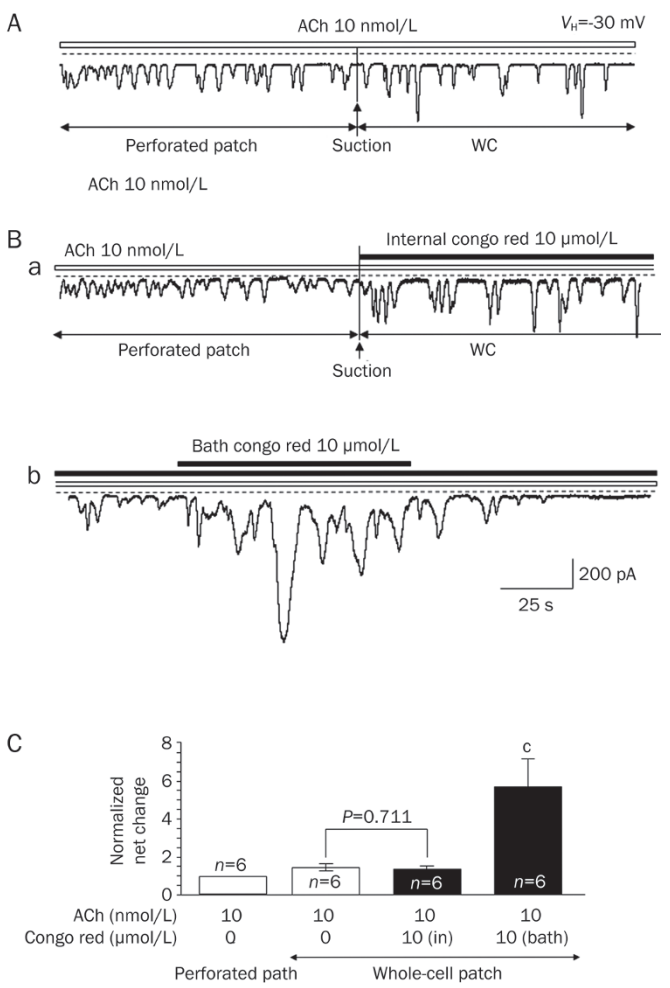

Figure 6. Effects of intracellular congo red on ACh-induced Ca^{2+} oscillations. (A) A representative trace showing the patch recording mode switch from perforated patch to whole-cell recording (without congo red in the pipette solution) in the presence of 10 nmol/L bath ACh. (B) The same recording mode switch with a recording electrode containing 10 μmol/L congo red (a), followed by bath application of 10 μmol/L congo red (b). (C) A summary of pooled data from 6A and 6B showing that, in contrast to extracellular application, intracellular application of congo red did not affect ACh-induced Ca^{2+} oscillations. Therefore, congo red potentiates ACh-induced Ca^{2+} oscillations via an extracellular target.

Comparison of the effects of congo red to 2-APB on ACh-induced Ca^{2+} oscillations

To explore the possible role of congo red in modulation of store-operated Ca^{2+} channels (SOCCs), we compared the effects of congo red to the effects of 2-APB, a SOCC blocker, on ACh-induced Ca^{2+} oscillations. Figure 7 demonstrates that both congo red (Figure 7A) and 2-APB (Figure 7B) potentiated ACh-induced Ca^{2+} oscillations in the same manner, suggesting that congo red may modulate ACh-induced Ca^{2+} oscillations through the blockade of SOCCs.

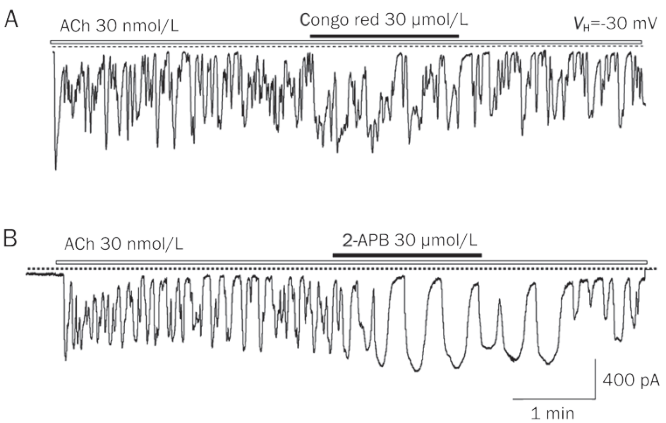

Figure 7. Effects of 2-APB, a well-known SOCC blocker, on ACh-induced Ca^{2+} oscillations. (A) A representative typical trace showing that application of 30 μmol/L congo red enhanced ACh (30 nmol/L)-induced Ca^{2+} oscillations. (B) A representative typical trace showing that application of 30 μmol/L 2-APB enhanced ACh-induced Ca^{2+} oscillations. Both congo red and 2-APB potentiated ACh-induced Ca^{2+} oscillations in the same manner, which suggests that congo red may block SOCCs in pancreatic acinar cells.

Discussion

Congo red was first synthesized in 1883, and it is commonly used as a histological stain for amyloid detection because congo red binds specifically to fibrillary proteins enriched in the β-sheet conformation. This feature, in addition to its small molecular size and ease of penetration of the blood-brain barrier, make congo red useful as a molecular probe for both the antemortem and *in vivo* visualization and quantification of brain amyloid[1, 2, 5, 6]. Interestingly, emerging studies demon-

strate that congo red can interfere with the processes of protein misfolding and aggregation and stabilizes native protein monomers or partially folded intermediates while reducing the concentration of more toxic protein oligomers[4, 9]. It has been reported that congo red is able to block Aβ aggregation and toxicity in rat hippocampal neuron culture[20], HeLa and PC12 cells and in human macrophage culture[21, 22]. In addition, congo red can inhibit amyloid toxicity by the blockade of Ca^{2+} permeable amyloid-formed ion channels[6, 7]. Congo red has demonstrated neuroprotective effects in a variety of models of neurodegenerative disorders, such as Alzheimer's, Parkinson's, Huntington's and prion disease[23]. Therefore, understanding the underlying mechanisms of congo red will be instructive for the design of future compounds to monitor and treat neurodegenerative diseases. However, other than binding to fibrillary proteins in a β-sheet conformation, little is known regarding congo red's effects on cell biology. We assessed whether congo red modulates intracellular Ca^{2+} signaling due to its importance as an intracellular signaling molecule that can exert allosteric regulatory effects on many enzymes and proteins[24]. The novel and important finding of this study is that congo red significantly enhances low concentrations of ACh-induced Ca^{2+} oscillations.

The targets that mediate the congo red-induced potentiation of intracellular Ca^{2+} signaling are still unclear. The underlying signaling pathway includes ACh activation of the muscarinic receptor, which then activates phospholipase C (PLC). PLC hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to form IP_3 and diacylglycerol (DAG), two classical second messengers[13]. DAG activates protein kinase C (PKC), while IP_3 diffuses into the ER and binds to the IP_3 receptor, a Ca^{2+} channel. This results in Ca^{2+} release from the ER to the cytosolic region in a process called IP_3-induced Ca^{2+} release, which can further trigger Ca^{2+}-induced Ca^{2+} release via a ryanodine receptor-sensitive Ca^{2+} pool[12, 14]. The resulting depletion of Ca^{2+} from the ER leads to activation of both ER Ca^{2+} pumps and plasma membrane SOCCs[15]. Thus, ACh induces repetitive Ca^{2+} oscillations in pancreatic acinar cells via these mechanisms, any of which could be a target of congo red activity. We observed that congo red exposure significantly enhanced IP_3-induced Ca^{2+} oscillations, suggesting that muscarinic receptor-PLC signaling is not necessary for the congo red-induced potentiation of Ca^{2+} oscillations. We also used Ca^{2+} imaging to demonstrate that congo red enhanced intracellular Ca^{2+} oscillations, suggesting that Ca^{2+}-dependent Cl^- channels are not involved. However, intracellular application of congo red failed to alter ACh-induced Ca^{2+} oscillations, which clearly suggests that this effect of congo red involves extracellular, but not intracellular, sites.

If congo red's effect on Ca^{2+} oscillations in pancreatic acinar cells is extracellular, and neither muscarinic receptors nor Ca^{2+}-dependent Cl^- channels are necessary, then SOCCs could be a potential target of congo red. In fact, we showed that the SOCC antagonist 2-APB potentiated ACh-induced Ca^{2+} oscillations in a manner similar to congo red (Figure 7). Our results, combined with the evidence that congo red exhibits the ability to block Ca^{2+} permeable channels formed by amyloid[6, 7], suggest that congo red might block SOCC channels in pancreatic acinar cells, wherein low Ca^{2+} concentration can trigger SOCC channel opening to refill the intracellular Ca^{2+} pool. Thus, under resting conditions in the absence of ACh, when Ca^{2+} pools are full and SOCC channels are mainly closed, congo red would not induce Ca^{2+} responses. However, when Ca^{2+} pools are partially emptied by the persistent presence of low concentrations of ACh and SOCC channels are open, congo red could promote Ca^{2+} release as a homeostatic response. In the presence of high concentrations of ACh, Ca^{2+} pools would be depleted, so congo red would not be able to further enhance the Ca^{2+} response, even though SOC channels are fully opened.

In summary, we demonstrate the novel finding that congo red significantly potentiates agonist-induced Ca^{2+} oscillations in mouse pancreatic acinar cells. Congo red alone does not induce any detectable Ca^{2+} signaling, but potentiates Ca^{2+} oscillations during ACh activation. Congo red-induced enhancement of Ca^{2+} oscillations occurs at low (10–30 nmol/L), but not high (100–10000 nmol/L), concentrations of ACh, and only when applied extracellularly. Based on these data, we propose that congo red might block the SOCC channels and provide a putative explanation of how congo red modulates agonist-induced intracellular Ca^{2+} signaling. Considering that congo red has great potential to be developed as a novel therapeutic drug for treating various neurodegenerative diseases, our findings provide new insights into and improved understanding of the pharmacological mechanism of congo red, especially on intracellular Ca^{2+} signaling.

Acknowledgements

Part of this work was supported by Shantou University Seed Fund (Jie WU) and by Guangdong Science and Technology Foundation (Hai-yan WANG).

Author contribution

Ze-bing HUANG performed patch-clamp experiments and wrote the manuscript; Hai-yan WANG designed research; Na-na SUN performed Ca^{2+} imaging experiments; Jing-ke WANG performed Ca^{2+} imaging experiments; Meng-qin ZHAO performed experiments; Jian-xin SHEN designed experiments, analyzed data and wrote part of manuscript; Ming GAO performed experiments; Ronald P HAMMER Jr designed experiments and wrote and edited manuscript; Xue-gong FAN designed experiments and edited the manuscript; Jie WU designed experiments, analyzed data and wrote manuscript.

Supplementary information

Supplementary figure is available at the Acta Pharmacologica Sinica website.

References

1 Carter DB, Chou KC. A model for structure-dependent binding of Congo red to Alzheimer beta-amyloid fibrils. Neurobiol Aging 1998;

19: 37–40.
2 Inouye H, Kirschner DA. Alzheimer's beta-amyloid: insights into fibril formation and structure from Congo red binding. Subcell Biochem 2005; 38: 203–24.
3 Khurana R, Uversky VN, Nielsen L, Fink AL. Is Congo red an amyloid-specific dye? J Biol Chem 2001; 276: 22715–21.
4 Lendel C, Bolognesi B, Wahlström A, Dobson CM, Gräslund A. Detergent-like interaction of Congo red with the amyloid beta peptide. Biochemistry 2010; 49: 1358–60.
5 Wang CC, Huang HB, Tsay HJ, Shiao MS, Wu WJ, Cheng YC, *et al*. Characterization of Abeta aggregation mechanism probed by congo red. J Biomol Struct Dyn 2012: 30: 160–9.
6 Hirakura Y, Yiu WW, Yamamoto A, Kagan BL. Amyloid peptide channels: blockade by zinc and inhibition by Congo red (amyloid channel block). Amyloid 2000; 7: 194–9.
7 Kagan BL, Azimov R, Azimova R. Amyloid peptide channels. J Membr Biol 2004; 202: 1–10.
8 Lorenzo A, Yankner BA. Beta-amyloid neurotoxicity requires fibril formation and is inhibited by congo red. Proc Natl Acad Sci U S A 1994; 91: 12243–7.
9 Podlisny MB, Walsh DM, Amarante P, Ostaszewski BL, Stimson ER, Maggio JE, *et al*. Oligomerization of endogenous and synthetic amyloid beta-protein at nanomolar levels in cell culture and stabilization of monomer by Congo red. Biochemistry 1998; 37: 3602–11.
10 Petersen OH. Ca^{2+} signaling in pancreatic acinar cells: physiology and pathophysiology. Braz J Med Biol Res 2009; 42: 9-16.
11 Dolman NJ, Tepikin AV, Petersen OH. Generation and modulation of cytosolic Ca^{2+} signals in pancreatic acinar cells: techniques and mechanisms. Biochem Soc Trans 2003; 31: 947–9.
12 Thorn P, Lawrie AM, Smith PM, Gallacher DV, Petersen OH. Ca^{2+} oscillations in pancreatic acinar cells: spatiotemporal relationships and functional implications. Cell Calcium 1993; 14: 746–57.
13 Petersen OH, Gallacher DV, Wakui M, Yule DI, Petersen CC, Toescu EC. Receptor-activated cytoplasmic Ca^{2+} oscillations in pancreatic acinar cells: generation and spreading of Ca^{2+} signals. Cell Calcium 1991; 12: 135–44.
14 Petersen OH. Stimulus-secretion coupling: cytoplasmic calcium signals and the control of ion channels in exocrine acinar cells. J Physiol 1992; 448: 1–51.
15 Csutora P, Zarayskiy V, Peter K, Monje F, Smani T, Zakharov SI, *et al*. Activation mechanism for CRAC current and store-operated Ca^{2+} entry: calcium influx factor and Ca^{2+}-independent phospholipase A2beta-mediated pathway. J Biol Chem 2006; 281: 34926–35.
16 Walsh CM, Chvanov M, Haynes LP, Petersen OH, Tepikin AV, Burgoyne RD. Role of phosphoinositides in STIM1 dynamics and store-operated calcium entry. Biochem J 2010; 425: 159–68.
17 Wu J, Takeo T, Kamimura N, Wada J, Suga S, Hoshina Y, *et al*. Thimerosal modulates the agonist-specific cytosolic Ca^{2+} oscillatory patterns in single pancreatic acinar cells of mouse. FEBS Lett 1996; 390: 149–52.
18 Wu J, Kamimura N, Takeo T, Suga S, Wakui M, Maruyama T, *et al*. 2-Aminoethoxydiphenyl borate modulates kinetics of intracellular $Ca^{(2+)}$ signals mediated by inositol 1,4,5-trisphosphate-sensitive $Ca^{(2+)}$ stores in single pancreatic acinar cells of mouse. Mol Pharmacol 2000; 58: 1368–74.
19 Wu J, Takeo T, Suga S, Kanno T, Osanai T, Mikoshiba K, *et al*. 2-aminoethoxydiphenyl borate inhibits agonist-induced Ca^{2+} signals by blocking inositol trisphosphate formation in acutely dissociated mouse pancreatic acinar cells. Pflugers Arch 2004; 448: 592–5.
20 Burgevin MC, Passat M, Daniel N, Capet M, Doble A. Congo red protects against toxicity of beta-amyloid peptides on rat hippocampal neurones. Neuroreport 1994; 5: 2429–32.
21 Pollack SJ, Sadler II, Hawtin SR, Tailor VJ, Shearman MS. Sulfated glycosaminoglycans and dyes attenuate the neurotoxic effects of beta-amyloid in rat PC12 cells. Neurosci Lett 1995; 184: 113–6.
22 Gellermann GP, Ullrich K, Tannert A, Unger C, Habicht G, Sauter SR, *et al*. Alzheimer-like plaque formation by human macrophages is reduced by fibrillation inhibitors and lovastatin. J Mol Biol 2006; 360: 251–7.
23 Aulić S, Bolognesi ML, Legname G. Small-Molecule Theranostic Probes: A Promising Future in Neurodegenerative Diseases. Int J Cell Biol 2013; 2013: 150952.
24 Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol 2000; 1: 11–21.