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Effect of xanthine oxidase-catalyzed reactive oxygen species generation on secretagogue-evoked calcium mobilization in mouse pancreatic acinar cells

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Abstract

In the present study we have employed fura-2 loaded isolated mouse pancreatic acinar cells to monitor the effect that xanthine oxidase (XOD)-catalyzed reactive oxygen species generation presents on Ca^{2+} mobilization by the secretagogue cholecystokinin octapeptide (CCK-8). Our results show that perfusion of pancreatic acinar cells with CCK-8 at a physiological concentration (20 pM) induced low frequency oscillations in intracellular free calcium concentration ($[Ca^{2+}]_i$) at a rate of 1 per minute; this oscillatory pattern was completely inhibited by the introduction in the perifusion medium of 20 mU/mL XOD to generate reactive oxygen species. In addition, perfusion of pancreatic acinar cells with 20 mU/mL XOD in the absence of extracellular calcium led to a transient increase in $[Ca^{2+}]_i$, that blocked the initiation of the Ca^{2+} signals in response to 20 pM CCK-8. Similarly, XOD was also able to block acetylcholine evoked Ca^{2+} spikes. However, reactive oxygen species had no effect either on Ca^{2+} extrusion or on re-uptake into intracellular stores, but CCK-8-evoked Ca^{2+} entry was reduced by XOD. In conclusion, our results show that XOD-evoked reactive oxygen species generation leads to a reduction either of Ca^{2+} mobilization, following stimulation of pancreatic acinar cells with the Ca^{2+}-mobilizing agonists CCK-8 and acetylcholine, and Ca^{2+} influx evoked by CCK-8 depletion of intracellular stores. The possible XOD inhibitory mechanism on Ca^{2+} mobilization by agonists is discussed.

Keywords: Ca^{2+}; Exocrine pancreas; Cholecystokinin; Oxidative stress; Xanthine oxidase

1. Introduction

It is well known that in a majority of cellular types, including the exocrine pancreas, initiation of biological responses involves phospholipid metabolism via the hydrolysis of membrane-bound PIP_2 to generate IP_3, and DAG [1]. Focusing onto Ca^{2+} metabolism, IP_3 is able to release calcium from intracellular nonmitochondrial stores [2]. To contribute as well to an increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), following stimulation of cells with Ca^{2+}-mediated agonists, a Ca^{2+} influx pathway is activated [3]. The role of ROS on Ca^{2+} homeostasis has been object of study during the past years. ROS can be produced in the course of different physiological processes and can react with a large variety of easily oxidizable cellular components [4–9], leading to induction of biological activities. Thus, oxidation-reduction (redox) reactions have gained attention as important chemical processes that can regulate signal transduction. In this sense, the action of oxidants, or oxidants-derived products, can provoke breakdown of membrane-bound phospholipids, such as phosphatidylinositol, leading to release of second messenger molecules like arachidonic acid, diacylglycerol, and phosphoinositide [9]. In additon, ROS can act to cause damage to both cell membrane [10] and intracellular organelles membranes [7]. Although careful attention has been paid to the Ca^{2+}-mobilizing effects of ROS and their involvement as pathogenic factors and apoptosis in different tissues, few evidence about the effect of oxidative stress on hormone-evoked Ca^{2+} signaling has been shown.

The source(s) of Ca^{2+} release in response to ROS generation is under current study, and evidence suggests that

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† *Abbreviations:* ACh, acetylcholine; Ca^{2+}, calcium; $[Ca^{2+}]_i$, intracellular free calcium concentration; CCK-8, cholecystokinin octapeptide; DAG, diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; PIP_2, phosphatidylinositol 4,5-bisphosphate; PLC, phospholipase C; ROS, reactive oxygen species; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase; TPS, thapsigargin; and XOD, xanthine oxidase.

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ROS evoke Ca^{2+} release from intracellular stores of different nature such as mitochondria [11], ryanodine-sensitive stores [12], IP_3-sensitive stores [13], and/or thapsigargin-sensitive stores [14]. It is well known that following an increase in $[Ca^{2+}]_i$, its value returns to prestimulation levels by different mechanisms involving Ca^{2+} extrusion toward the extracellular medium [15,16] and a re-uptake into intracellular stores [17,18]. Regarding Ca^{2+} re-uptake into intracellular stores, it has been published that SERCA pumps are inactivated by ROS in HEK293 cells [19], synaptosomes [20], oocytes [21], vascular tissue, [22], cardiomyocytes [23], or skeletal muscle [24]. On the other hand, it has been proposed that ROS reduce plasma membrane Ca^{2+} pump activity [20], therefore decreasing Ca^{2+} extrusion from the cell. Differential implications have been also proposed for ROS on Ca^{2+} influx, stimulating [14], inhibiting [25] or without effect on Ca^{2+} influx [23]. Thus, controversial results exist regarding the effect of ROS generation on Ca^{2+} homeostasis in different tissues including the exocrine pancreas. Although most of the studies have been carried out in a diversity of cellular types and tissues, little is known about the effect of ROS generation on Ca^{2+} mobilizing effect by secretagogues in the exocrine pancreas.

In the present study we have employed fura-2 loaded isolated mouse pancreatic acinar cells to monitor the effect that XOD-catalyzed ROS generation presents on Ca^{2+} mobilization by the secretagogue cholecystokinin. Our results show that XOD-evoked ROS generation leads to a reduction of Ca^{2+} mobilization when pancreatic acinar cells were stimulated with the Ca^{2+}-mobilizing agonist CCK, whereas Ca^{2+} influx into pancreatic acinar cells is partially inhibited in the presence of ROS. The observed inhibition of receptor-activated release of Ca^{2+} from internal stores together with the partial inhibition of Ca^{2+} influx, could be some of the mechanisms underlying the action of ROS in oxidative stress, that lead to the impairment of physiological processes therefore leading to cell damage and dysfunction.

2. Materials and methods

2.1. Animals and chemicals

Adult male Swiss mice were used throughout this study. Fura-2/AM was obtained from Molecular Probes (Eugene, OR, USA) and thapsigargin from Alomone Labs (Israel). All other materials used were obtained from Sigma (Spain).

2.2. Cell isolation procedure

Animals were sacrificed by cervical dislocation, the pancreas was rapidly removed and the acinar cells were isolated as described previously [26]. Briefly the pancreas was incubated in the presence of collagenase for 10 min at 37°. This enzymatic digestion of the tissue was followed by gently pipetting the cell suspension through tips of decreasing diameter for mechanical dissociation of the cells. After centrifugation cells were resuspended in a buffer without collagenase. With this isolation procedure single cells as well as small clusters consisting of up to five cells were obtained. All experiments were performed at room temperature (23–25°).

2.3. Dye loading

Freshly isolated mouse pancreatic acinar cells were loaded with fura-2 acetoxymethyl ester 4 μM at room temperature for 40 min following previously stablished methods [17]. Changes in fluorescence emitted by this fluorophore reflect changes in $[Ca^{2+}]_i$ [27]. The cells were kept at 4° until use and the experiments were performed within the next 4 h.

2.4. Fluorescence determinations and cytosolic Ca^{2+} measurements

For monitoring Ca^{2+}-dependent fluorescence signals, aliquots of dye-loaded cells were placed onto a coverslip attached to the bottom of a perfusion chamber on the stage of an epifluorescence inverted microscope (Nikon diaphot T200, Melville, NY, USA). Perfusion (~1.5 mL/min) at room temperature was started after a 5-min period to allow spontaneous attachment of the cells to the coverslip. No coating treatment was necessary to immobilize the cells. The cells were continuously superfused with a Na-HEPES buffer containing (in mM): 140 NaCl, 4.7 KCl, 1.3 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 glucose (pH adjusted to 7.4) and alternatively excited with light from a short arc xenon lamp at 340/380 nm employing a computer-controlled filter wheel (Lambda 10–2, Shutter Instruments Ltd.). Fluorescence emission at 505 nm was detected by using a cooled digital CCD camera (C-4880, Hamamatsu Photonics, Hamamatsu City, Japan) and recorded by using dedicated software (Argus-HisCa, Hamamatsu Photonics). Results are expressed as the ratio fluorescence emmited at both excitation wavelengths.

2.5. Reactive oxygen species (ROS) generation

Oxygen free radicals, mainly hydroxyl radicals, were generated in a system by which XOD oxidizes hypoxanthine to xanthine and further to uric acid [12]

2.6. Statistical analysis

Where expressed values are means ± SEM. Statistical significance was calculated by Student's *t*-test. Only *P* values under 0.05 were considered as statistically significant.

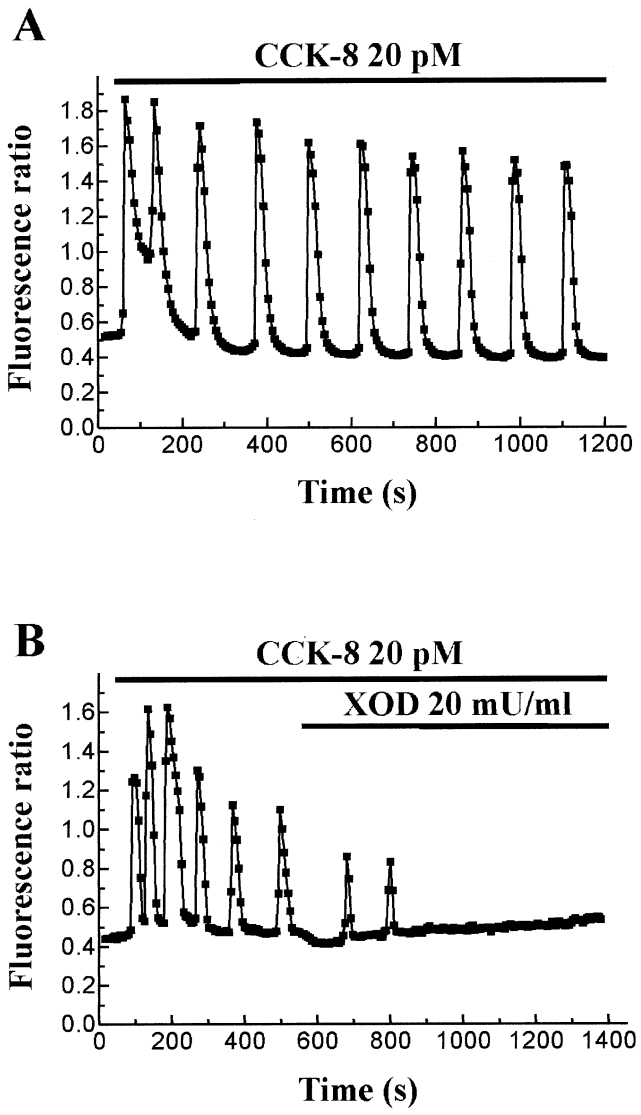

Fig. 1. Effect of XOD on CCK-8-evoked oscillations in $[Ca^{2+}]_i$. (A) Stimulation of pancreatic acinar cells with the secretagogue cholecystokinin at a physiological concentration (20 pM) in the absence of extracellular Ca^{2+} (medium containing 1 mM EGTA), induced low frequency oscillations in $[Ca^{2+}]_i$ at a rate of 1/min in all cells examined (n = 54 cells/4 animals). (B) When in the presence of CCK-8 20 pM, XOD (20 mU/mL) was introduced in the perfusion medium to generate ROS, the oscillatory pattern of $[Ca^{2+}]_i$ in response to CCK-8 was strongly inhibited (n = 115 cells/9 animals). Experiments were performed in a Ca^{2+} free medium containing 1 mM EGTA.

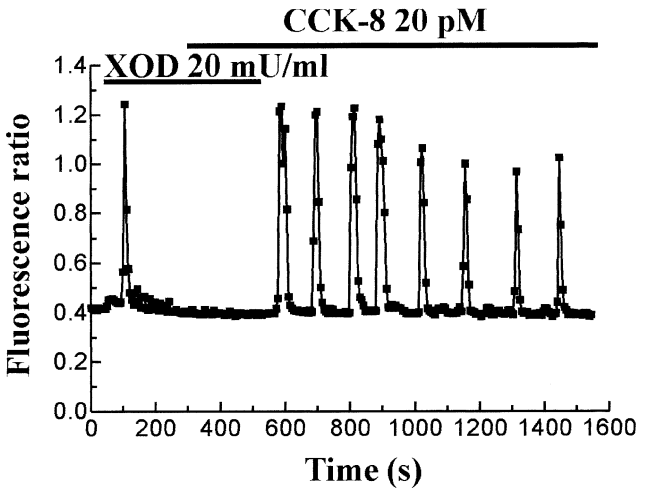

Fig. 2. Inhibition by XOD of CCK-8-induced responses in $[Ca^{2+}]_i$. Perfusion of pancreatic acinar cells with XOD in the absence of extracellular Ca^{2+} (medium containing 1 mM EGTA) led to a transient release of Ca^{2+} from intracellular stores and the initiation of the Ca^{2+} signals in response to secondary application of CCK-8 20 pM was inhibited. Following removal of XOD from the perfusion medium, an oscillatory pattern in $[Ca^{2+}]_i$ could be observed, typical of a response to the physiological concentration of CCK-8. Traces are representative of such 69 cells taken from 6 animals.

3. Results

3.1. Effect of XOD on secretagogue-evoked Ca^{2+} release from intracellular stores

As expected, perfusion of pancreatic acinar cells with the secretagogue cholecystokinin at a physiological concentration (20 pM) in a Ca^{2+} free medium (1 mM EGTA), induced low-frequency oscillations in $[Ca^{2+}]_i$ at a rate of 1 per minute in all cells examined (Fig. 1A). When in the presence of CCK-8 20 pM, XOD (20 mU/mL) was introduced in the perfusion medium to generate ROS, the oscillatory pattern of $[Ca^{2+}]_i$ in response to CCK-8 was strongly inhibited in all cells studied (Fig. 1B). Furthermore, as shown in Fig. 2, perfusion of pancreatic acinar cells with XOD in the absence of extracellular Ca^{2+} (medium containing 1 mM EGTA) led to a transient release of Ca^{2+} from intracellular stores. ROS-evoked Ca^{2+} response reached a maximal value of 293 ± 43.4 nM over a basal $[Ca^{2+}]_i$ of 103.9 ± 8.7 nM (n = 51 cells/5 animals). In addition, the observed Ca^{2+} release evoked by ROS inhibited the initiation of the Ca^{2+} signals in response to CCK-8 20 pM. Following removal of XOD from the perfusion medium, an oscillatory pattern in $[Ca^{2+}]_i$ could be observed, typical of a response to the physiological concentration of CCK-8 (Fig. 2).

We also performed experiments with another Ca^{2+}-mediated secretagogue, ACh 10 μM, in a Ca^{2+} free medium too (1 mM EGTA). Application of short pulses (10–15 s) of 10 μM ACh, evoked spikes of Ca^{2+} release from intracellular stores as shown in Fig. 3A. The amplitude of the ACh-evoked Ca^{2+} spike remained nearly unchanged until the end of the experiment. At this point, a longer stimulation of the cells with the neurotransmitter led to a larger Ca^{2+} response, therefore showing that the intracellular Ca^{2+} stores were not completely depleted yet. When the same protocol was tested in the presence of ROS, generated by XOD-catalyzed reaction of hypoxanthine to xanthine and uric acid, the amplitude of the Ca^{2+} spikes in response to short applications of ACh was progressively decreased till nearly disappear (Fig. 3B). At the end of the experiment, a

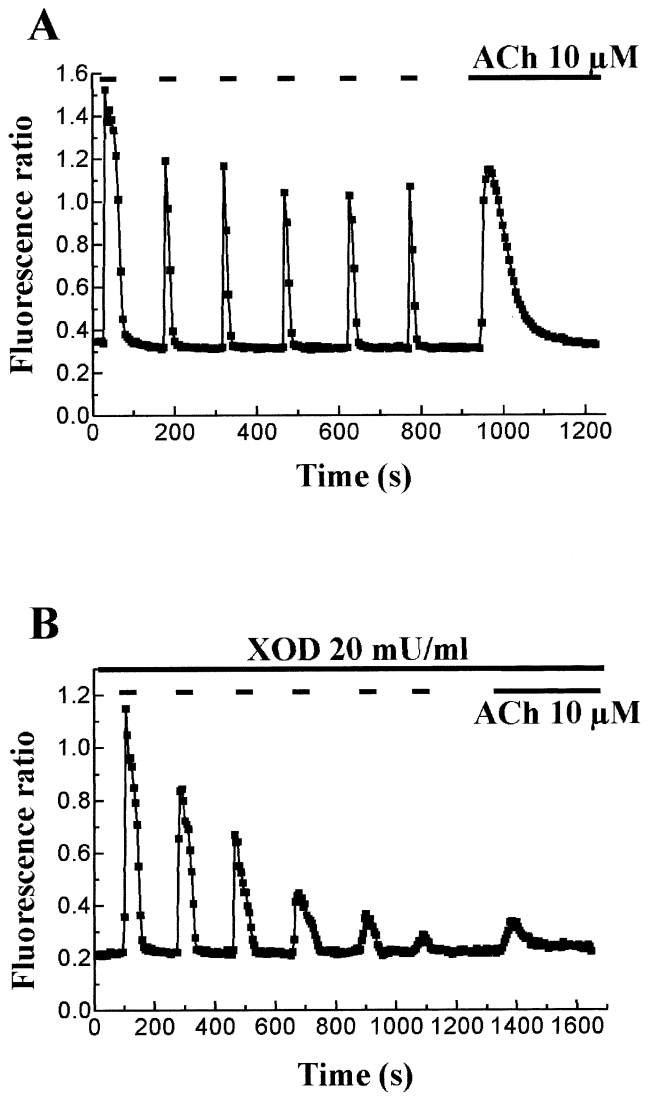

Fig. 3. Effect of XOD on ACh-induced Ca^{2+} release from intracellular stores. (A) Application of short pulses (10–15 s) of 10 μM ACh in a Ca^{2+} free medium (1 mM EGTA) evoked transients of Ca^{2+} release from intracellular stores, whose amplitude remained nearly unchanged until the end of the experiment. At this point, a longer stimulation of the cells with the neurotransmitter led to a larger Ca^{2+} response (n = 44 cells, 4 animals). (B) When ACh was tested in the presence of XOD-evoked ROS generation, the amplitude of the Ca^{2+} spikes in response to short applications of ACh was progressively decreased till nearly disappear. At the end of the experiment, a longer stimulation with ACh released a small amount of Ca^{2+} in contrast to a larger response observed when ACh was tested alone (n = 91 cells, 6 animals).

longer stimulation with ACh released a small amount of Ca^{2+} in contrast to a larger response observed in the control experiments, where ACh was tested alone (Fig. 3A).

3.2. Effect of ROS on Ca^{2+} extrusion

Following Ca^{2+} release from intracellular stores a plasma membrane Ca^{2+}-ATPase extrudes Ca^{2+} from the cytosol towards the extracellular medium [15,16]. To test whether the inhibitory role of ROS on CCK-8-evoked oscillations in $[Ca^{2+}]_i$ is the result of an increased extrusion of Ca^{2+} towards the extracellular medium, we performed a set of experiments in which the cells were stimulated with a maximal concentration of CCK-8 (10 nM) plus thapsigargin (TPS, 1 μM), a known inhibitor of the SERCA pump [28,29] in the absence of extracellular Ca^{2+} (1 mM EGTA). This treatment leads to the depletion of intracellular Ca^{2+} stores and avoids reuptake into stores; thus, under these conditions, the decrease of $[Ca^{2+}]_i$ reflects Ca^{2+} extrusion towards the extracellular medium. When XOD was present in the perfusion medium no statistically significant differences could be observed in $[Ca^{2+}]_i$ decay compared to the control experiments where CCK-8 and TPS were tested alone (Fig. 4A).

3.3. Effect of ROS on Ca^{2+} re-uptake

Another possibility for this observed effect of ROS on $[Ca^{2+}]_i$ is that ROS were activating the Ca^{2+}-ATPase of the ER. To clarify this, we performed a set of experiments in which the re-uptake of Ca^{2+} was assayed. To isolate this component we used a short pulse of the neurotransmitter ACh, which stimulation can be suddenly interrupted by fast washout with atropine. This maneuver induces a fast recovery of $[Ca^{2+}]_i$ towards the resting levels due to Ca^{2+} reuptake into the stores [30]. To exclude a minor contribution of Ca^{2+} extrusion we included in the perfusion medium 2 mM La^{3+}, an inhibitor of the plasma membrane calcium ATPase activity [31]. Under our experimental conditions no differences could be observed in the slope of the curve when XOD (20 mU/mL) was included in the perfusion medium compared to the control experiments in which 10 μM ACh was washed out only with 100 μM atropine plus 2 mM $LaCl_3$ (Fig. 4B).

3.4. Effect of ROS on Ca^{2+} influx

It is well known that following Ca^{2+} release from intracellular stores a capacitative Ca^{2+} entry pathway is activated [3]. As a part of the effects of ROS generation on Ca^{2+} homeostasis, Ca^{2+} influx might be affected. As shown in figure 5, perfusion of pancreatic acinar cells with a maximal concentration of CCK-8 (10 nM) in the absence of extracellular Ca^{2+} (1 mM EGTA) led to a typical increase in $[Ca^{2+}]_i$ that depleted intracellular Ca^{2+} stores. Following reintroduction of Ca^{2+} in the perfusion medium an increase in $[Ca^{2+}]_i$ was observed, indicating Ca^{2+} influx into pancreatic acinar cells. If during Ca^{2+} entry phase, 20 mU/mL XOD was included in the perfusion medium, a decrease in the fluorescence was observed, what we considered as an inhibition of Ca^{2+} entry into the cells. This inhibition of Ca^{2+} was not complete, although it was statistically significant ($P < 0.05$). The calculated inhibition of Ca^{2+} influx in the presence of XOD in the extracellular medium was of a 13.4 ± 4.4% (n = 58 cells/5 animals).

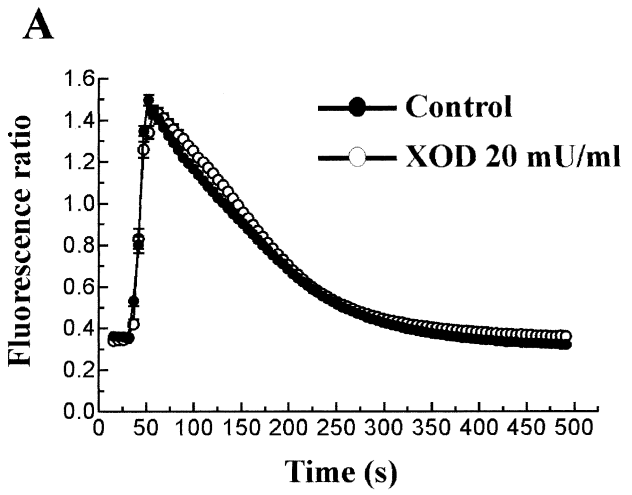

Fig. 4. Effect of XOD-evoked ROS generation on Ca^{2+} extrusion and reuptake. (A) $[Ca^{2+}]_i$ decay following release of Ca^{2+} by perfusion of pancreatic acinar cells with CCK-8 (10 nM) plus TPS (1 μM) (○), in the presence of 20 mU/mL XOD and in the absence of extracellular Ca^{2+} (1 mM EGTA), did not differ statistically from the control experiments where CCK-8 and TPS were applied alone (●). Data show mean values of ratio fluorescence ± SEM under each treatment. Traces are representative of 192 such cells taken from 11 animals. (B) When Ca^{2+} re-uptake into intracellular stores was assayed, no statistically significant differences could be observed in the slope of the curve in the presence of XOD (20 mU/mL) in the perfusion medium (□), compared to the absence of XOD (control experiments) (●). To isolate this component, we applied a short pulse of the neurotransmitter acetylcholine (ACh), which stimulation can be suddenly interrupted by fast washout with atropine. This maneuver induces a fast recovery of $[Ca^{2+}]_i$ toward the resting levels due to Ca^{2+} reuptake into the stores. Two mM La^{3+}, an inhibitor of the plasma membrane calcium ATPase activity, was introduced in the perfusion medium to exclude a minor contribution of Ca^{2+} extrusion. For the comparisons of treatments, each individual record was normalized to the maximum response in each experiment. Data show mean values of ratio fluorescence ± SEM under each treatment, and are representative of 109 such cells taken from 8 animals.

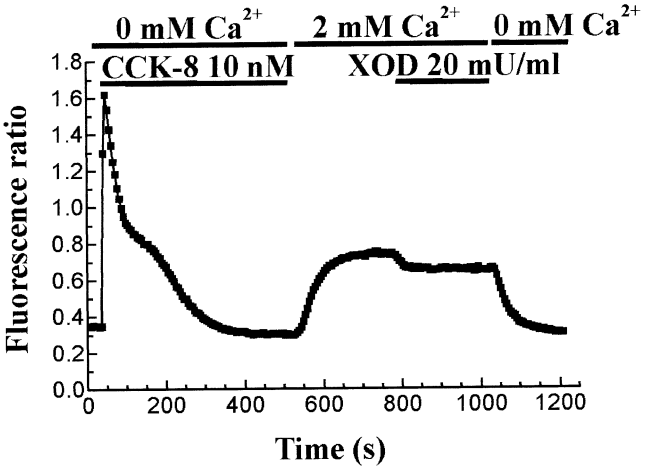

Fig. 5. Effect of ROS on Ca^{2+} influx in pancreatic acinar cells. Depletion of intracellular Ca^{2+} stores by stimulation of pancreatic acinar cells with 10 nM CCK-8 in the absence of extracellular Ca^{2+} (medium containing 1 mM EGTA), activated Ca^{2+} influx that was partially inhibited by the presence of XOD in the perfusion medium. At the end of the experiment, exclusion of Ca^{2+} from the extracellular medium led to a larger decrease of fluorescence, as index of total absence of Ca^{2+} entry. Traces are representative of 58 such cells taken from 5 animals.

4. Discussion

In the present study we have employed fura-2 loaded isolated mouse pancreatic acinar cells to monitor the effect that XOD-catalyzed ROS generation presents on Ca^{2+} mobilization by the secretagogue CCK-8. Our results show that XOD is able to attenuate the oscillations in $[Ca^{2+}]_i$ evoked by the Ca^{2+} mobilizing agonists CCK-8 and ACh, and reduces store-depletion-evoked Ca^{2+} entry.

The results show that the oscillatory pattern in $[Ca^{2+}]_i$ in response to a physiological concentration of the secretagogue CCK-8, was completely inhibited by the introduction in the perfusion medium of XOD to generate ROS (Fig. 1B). ROS effect on basal $[Ca^{2+}]_i$ has been previously described in rat pancreatic acinar cells [12]. The authors show that ROS generation by XOD-catalyzed reaction releases Ca^{2+} from intracellular stores. However, to our knowledge, the inhibitory effect of ROS on CCK-8-evoked changes in $[Ca^{2+}]_i$ has not been reported yet. It is well known that initiation of Ca^{2+} signals in the exocrine pancreas, as well as in many other cellular types, depends on the generation of IP_3 that releases Ca^{2+} from intracellular non-mitochondrial Ca^{2+} stores [2]. In our conditions, ROS could be somehow blocking IP_3 effect on the release of Ca^{2+} from intracellular stores. Further characterization of the inhibitory action of ROS on Ca^{2+} mobilization by IP_3 generation can be seen in Fig. 3B. In this set of experiments, ACh evoked spikes of Ca^{2+} release from intracellular stores were progressively decreased until nearly disappear in the presence of XOD-evoked ROS generation. Thus, enough evidence supports ROS inhibitory action on Ca^{2+} mobilization by agonists. On the other hand, it has been suggested that part of the effect of XOD-evoked ROS generation is mediated via

activation of PKC [32] and that PKC activation inhibits Ca^{2+} release from IP_3-sensitive stores [33]. If ROS are stimulating PKC this would explain the smaller release of Ca^{2+} by CCK-8 stimulation. Under our experimental conditions it could be possible that XOD-catalyzed ROS generation leads to inhibition of Ca^{2+} release from intracellular stores by inhibition of PLC, which is known to generate IP_3 from membrane bound phospholipids. From our observations, we cannot exclude the possibility that in the presence of ROS PLC could be inhibited, therefore leading to a lower concentration of IP_3 that would release less Ca^{2+} from the intracellular stores. However, evidence exists about a stimulation of PLC by peroxides in granulocytes. In this cell type, peroxides induced a transient elevation of $[Ca^{2+}]_i$ mediated by PLC activation [34]. If this effect was applicable to pancreatic acinar cells, this would rule out the inhibiton of Ca^{2+} release by a XOD action on PLC.

Following Ca^{2+} release from intracellular stores a plasma membrane Ca^{2+}-ATPase extrudes Ca^{2+} from the cytosol towards the extracellular medium [15,16]. It has been previously proposed that ROS reduce plasma membrane Ca^{2+} pump activity [11,20], therefore decreasing Ca^{2+} extrusion from the cell. Perfusion of pancreatic acinar cells with a maximal concentration of CCK-8 (10 nM) plus thapsigargin (TPS, 1 μM), a known inhibitor of the SERCA pump [28,29], in the absence of extracellular Ca^{2+}, leads to the depletion of intracellular Ca^{2+} stores and avoids Ca^{2+} re-uptake into stores; thus, under these conditions, the decrease of $[Ca^{2+}]_i$ reflects Ca^{2+} extrusion towards the extracellular medium. In our experimental conditions, when XOD was present in the perifusion medium no statistically significant differences could be observed in $[Ca^{2+}]_i$ decay compared to the experiments where CCK-8 and TPS were tested alone (Fig. 4A). Therefore, we can conclude that, although a clear inhibitory effect of ROS on Ca^{2+} mobilization exists, extrusion mechanisms are not affected.

It might be possible that ROS lead to a decrease in $[Ca^{2+}]_i$ stimulating the activity of the Ca^{2+}-ATPase located in the membrane of the ER. Although clear evidence about the inhibition of this pump by ROS has been published in different cellular types [19–24,35], studies in the exocrine pancreas are currently lacking. As it can be observed in Fig. 4B, no effect on Ca^{2+} re-uptake could be observed, therefore ruling out an effect of XOD-evoked ROS generation on the endoplasmic reticulum Ca^{2+}-ATPase.

Finally, it has been clearly reported that following Ca^{2+} release from intracellular stores a capacitative Ca^{2+} entry pathway is activated [3]; however, differential reports exist regarding the effect of ROS on Ca^{2+} influx (for references see introduction). In our conditions, as a part of the effects of ROS generation on Ca^{2+} homeostasis, Ca^{2+} influx might be affected. As it has been shown in the present paper, depletion of intracellular Ca^{2+} stores by perfusion of pancreatic acinar cells with a maximal concentration of CCK-8 in the absence of extracellular Ca^{2+} activated capacitative

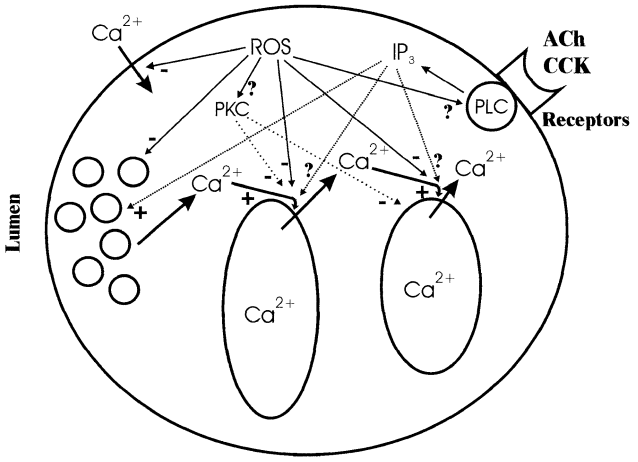

Fig. 6. Schematic representation of a pancreatic acinar cell and the mechanism of action of XOD-evoked ROS generation on Ca^{2+} homeostasis. Activation of PLC-linked secretagogue-receptors in the cell membrane, leads to an initial release of Ca^{2+} from IP_3-sensitive stores located in the luminal (granular) cell pole. Subsequently, Ca^{2+}-induced Ca^{2+} release from intracellular stores located in series throughout the cytosol occurs, probably with IP_3 cooperation too. In spite of its Ca^{2+} releasing effect in the absence of secretagogues, XOD-evoked ROS generation might somehow block IP_3 action to release Ca^{2+} from intracellular stores, or activate PKC, which has been shown to inhibit Ca^{2+} release from intracellular pools. Inhibition of PLC by ROS cannot be excluded. Although inhibition of Ca^{2+} extrusion towards the extracellular medium and re-uptake into intracellular stores could not be demonstrated, a partial (but significant) inhibition of Ca^{2+} influx was observed in the presence of ROS. (+: stimulation; −: inhibition).

Ca^{2+} influx into pancreatic acinar cells. Ca^{2+} entry was partially blocked in the presence of XOD-evoked ROS generation (Fig. 5).

In conclusion, our results show that XOD-evoked ROS generation leads to a reduction of Ca^{2+} mobilization following stimulation of pancreatic acinar cells with the Ca^{2+}-mobilizing agonists CCK-8 and ACh. Another observed effect is that ROS generation has little or no effect on other important mechanisms related to Ca^{2+} homeostasis like Ca^{2+} extrusion and reuptake into intracellular stores. However, Ca^{2+} influx, the mechanism that allows Ca^{2+} entry into the cell and subsequent replenishment of cytosolic stores, is partially inhibited in the presence of ROS. A proposed model for ROS effect on Ca^{2+} homeostasis can be seen in Fig. 6. The observed inhibition of receptor-activated release of Ca^{2+} from internal stores, together with the inhibition of Ca^{2+} influx, might be some of the mechanisms of action of ROS under oxidative stress that lead to the impairment of physiological processes, thereby leading to cell damage and dysfunction. Although most studies had been focused on the effect of ROS on basal $[Ca^{2+}]_{i,}$ few evidences had been reported until now regarding its action on secretagogue-evoked responses in the exocrine pancreas. Further investigations should be carried out to clearly identify the mechanism(s) of action of ROS on Ca^{2+} signaling in the exocrine pancreas.

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