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Original Article

Role of calcium-sensing receptor in regulating activation susceptibility of postovulatory aging mouse oocytes

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Abstract. The mechanisms underlying postovulatory oocyte aging (POA) remain largely unknown. The expression of the calcium-sensing receptor (CaSR) in mouse oocytes and its role in POA need to be explored. Our objective was to observe CaSR expression and its role in the susceptibility to activating stimuli (STAS) in POA mouse oocytes. The results showed that, although none of the newly ovulated oocytes were activated, 40% and 94% of the oocytes recovered 19 and 25 h after human chorionic gonadotropin (hCG) injection were activated, respectively, after ethanol treatment. The level of the CaSR functional dimer protein in oocytes increased significantly from 13 to 25 h post hCG. Thus, the CaSR functional dimer level was positively correlated with the STAS of POA oocytes. Aging *in vitro* with a CaSR antagonist suppressed the elevation of STAS, and cytoplasmic calcium in oocytes recovered 19 h post hCG, whereas aging with a CaSR agonist increased STAS, and cytoplasmic calcium of oocytes recovered 13 h post hCG. Furthermore, the CaSR was more important than the Na-Ca^{2+} exchanger in regulating oocyte STAS, and T- and L-type calcium channels were inactive in aging oocytes. We conclude that the CaSR is involved in regulating STAS in POA mouse oocytes, and that it is more important than the other calcium channels tested in this connection.
Key words: Calcium-sensing receptor, Na-Ca^{2+} exchanger, L-type calcium channel, Oocyte activation, Postovulatory oocyte aging

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In vivo [1, 2] and *in vitro* [2, 3] aging occurs when mammalian oocytes are not fertilized or activated after ovulation or *in vitro* maturation. Postovulatory oocyte aging (POA) has a marked adverse effect on embryo development [4, 5] and offspring health [6, 7]. POA have been envisioned as a major factor in reducing the population size of several threatened mammalian species [8]. Thus, in-depth research must be conducted on the mechanisms and control of POA to facilitate normal and assisted reproduction.

Studies have demonstrated that a spontaneous increase in susceptibility to activating stimuli (STAS) is one of the earliest manifestations of POA. For example, although only 3.4% of mouse oocytes recovered 11 h after human chorionic gonadotropin (hCG) injection were activated, activation rates increased to 88% in oocytes recovered 17.5 h post hCG following treatment with 8% ethanol for 6.5 min [9]. Lan *et al*. observed that, although 27% of mouse oocytes recovered 15 h after hCG injection were activated, 90% of the oocytes recovered 21 h after hCG injection were activated after treatment with 10% ethanol for 5 min [10]. Thus, studies on the mechanisms underlying increased STAS levels in aging oocytes are important for understanding the mechanisms of POA. However, the mechanisms underlying increased STAS levels in aging oocytes are largely unclear. Kong *et al*. [11] and Chen *et al*. [12] have reported that cytoplasmic calcium is correlated with STAS in aging mouse oocytes. Cui *et al*. [13] observed that cytoplasmic Ca^{2+} levels were increased in rat oocytes undergoing spontaneous activation during postovulatory aging. Furthermore, as intracellular Ca^{2+} oscillations are essential for the activation of mammalian oocytes during both fertilization and parthenogenetic activation [14–17], studies on the mechanisms of ooplasmic Ca^{2+} increases will contribute greatly to our understanding of the mechanism of STAS increases in aging oocytes.

The Ca^{2+}-sensing receptor (CaSR) is a G protein-coupled receptor that senses extracellular calcium ion levels. Elevations of extracellular Ca^{2+} induce a CaSR conformational change, which activates phospholipase C (PLC) through a G protein of Gqα type [18]. The activated PLC hydrolyzes the phosphatidylinositol 4,5-bisphosphate (PIP2), producing inositol 1,4,5-trisphosphate (IP3). The IP3 facilitates Ca^{2+} release from the endoplasmic reticulum by interacting with the IP3 receptors [19, 20]. Dell'Aquila *et al*. observed CaSR expression in maturing human oocytes but did not study its role in maturation [21]. De Santis *et al*. observed CaSR expression in maturing horse oocytes and found that it promotes maturation in the presence of high external Ca^{2+} [22]. Liu *et al*. reported that gonadotropin promotes porcine oocyte maturation by upregulating CaSR expression [23]. However, CaSR expression in aging oocytes has only been reported in rats [24]; the expression of CaSR in mouse oocytes and its role in the STAS of aging oocytes are largely unexplored.

The objective of the present study was to observe the expression of the CaSR in aging mouse oocytes and to verify its role in regulating STAS during POA.

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Materials and Methods

All animal care and handling procedures were approved by the Animal Care and Use Committee of Shandong Agricultural University, P. R. China (Permit number: SDAUA-2001-001). All chemicals and reagents were purchased from Sigma Chemical Co. unless otherwise indicated.

Oocyte recovery

Kunming strain mice were kept in a room with 14-h light:10-h dark cycles, with the dark period starting at 2000 h. Female mice, 8–10 weeks after birth, were induced to superovulate with 10 IU equine chorionic gonadotropin (eCG), followed by 10 IU hCG 48 h later. Both eCG and hCG were obtained from Ningbo Hormone Product Co., Ltd., Ningbo, China. Superovulated mice were euthanized at different time points after hCG injection, and the oviductal ampullae were broken to release the oocytes. After being dispersed and washed thrice in M2 medium, cumulus cells were denuded to prepare cumulus-denuded oocytes (DOs) by pipetting with a thin pipette in a drop of M2 medium containing 0.1% hyaluronidase.

Oocyte aging in vitro

The DOs were incubated for in vitro aging for 6 h in an aging medium containing different concentrations of a CaSR agonist/inhibitor or calcium channel blockers. The CZB medium [25] was used as the aging medium. Stock solutions were prepared by dissolving NPS-2143 (5 mM, SML0362), cinacalcet (1 mM, 1133977), nifedipine (200 mM, 481981), and ML218 (1.35 mM, SML0385) in DMSO. All stock solutions were stored in aliquots at –20°C and diluted to desired concentrations using the aging medium immediately before use. The aging culture was performed at 37°C under 5% CO_2 in humidified air using a 96-well culture plate with each well containing 200 µl of the aging medium and approximately 30 oocytes covered with mineral oil.

Oocyte activation treatment

At the end of the aging culture period, mouse oocytes were treated with ethanol to induce activation. Briefly, mouse oocytes were treated with 5% ethanol in M2 medium for 10 min at room temperature, washed thrice, and cultured in CZB medium for 6 h. This protocol for ethanol treatment was adopted because our preliminary experiment showed that, although oocytes recovered 13 h post hCG injection could not be activated well, > 90% of oocytes recovered 25 h post hCG injection were activated with this protocol. At the end of the culture period, oocytes were observed under a microscope for activation. Only oocytes that had one or two pronuclei or two cells, each with a nucleus, were considered activated.

Cytoplasmic calcium measurement

The DOs were incubated in the loading medium at room temperature for 20 min to load the Ca^{2+} probe. The loading medium was HCZB medium that contained 1 µM Fura-2 AM and 0.02% pluronic F-127. The HCZB medium was composed of 81.62 mM NaCl, 4.83 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$, 5 mM $NaHCO_3$, 20 mM HEPES, 1.7 mM $CaCl_2$•$2H_2O$, 31.3 mM sodium lactate, 0.27 mM sodium pyruvate, 0.11 mM EDTA, 1 mM glutamine, 5 g/l bovine serum albumin (BSA), 0.06 g/l penicillin, and 0.05 g/l streptomycin. After loading, DOs were transferred into a drop of HCZB medium in a Fluoro dish (FD35-100, World Precision Instruments, Sarasota, FL, USA) covered with mineral oil and observed using a Leica DMI6000 inverted microscope (Leica Germany, Wetzlar, Germany) at 37.5°C. A Fura 2 fluorescence module was used for excitation, and a Leica LAS-AF calcium imaging module was used to calculate the F340/380 ratio, which represented the concentration of cytoplasmic calcium. The DOs were monitored for 5 min to record the baseline F340/380 ratio and then treated for 10 min with 5% ethanol. Following ethanol stimulation, we monitored the oocytes in HCZB for 40 min to record the F340/380 ratio.

Immunofluorescence microscopy

If not mentioned otherwise, all procedures were conducted at room temperature and the DOs were washed thrice with M2 medium between treatments. DOs were (i) fixed for at least 30 min with 3.7% paraformaldehyde in PHEM buffer (60 mM Pipes, 25 mM Hepes, 10 mM EGTA, and 4 mM $MgSO_4$, pH 7.0); (ii) treated for 1–2 seconds with 0.25% protease in M2 to remove zona pellucida; (iii) permeabilized for 5 min with 0.1% Triton X-100 in PHEM; (iv) blocked for 1 h in PHEM containing 3% BSA; (v) incubated at 4°C overnight with mouse monoclonal anti-CaSR (IgG, 1:200, Abcam, Cambridge, UK, ab19347) in 3% BSA in M2 medium; (vi) incubated for 1 h with Cy3-conjugated goat-anti-mouse IgG (1:800, Jackson ImmunoResearch, West Grove, PA, USA, 115-005-003) in 3% BSA in M2; (vii) incubated for 10 min with 10 µg/ml Hoechst 33342 (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) in M2. Negative control samples in which the primary antibody was omitted were also used. The DOs were mounted on glass slides and observed under a Leica laser scanning confocal microscope (TCS SP2). Blue diode (405 nm) and helium/neon (He/Ne; 543 nm) lasers were used to excite Hoechst and Cy3, respectively. Fluorescence was detected using 420–480 nm (Hoechst) and 560–605 nm (Cy3) bandpass emission filters, and the captured signals were recorded as blue and red, respectively. The relative CaSR content was quantified by measuring the fluorescence intensity. For each experimental series, all high-resolution z-stack images were acquired using identical settings. The relative intensities of the raw images were measured using the Image-Pro Plus software (Media Cybernetics Inc., Silver Spring, MD, USA) under fixed thresholds across all slides.

Western blot analysis

We placed 200 DOs in a 1.5-ml microfuge tube that contained 20 µl sample buffer (20 mM Hepes, 100 mM KCl, 5 mM $MgCl_2$, 2 mM DTT, 0.3 mM PMSF, and 3 mg/ml leupetin, pH 7.5) and froze the sample at –80°C until use. Then, 5 µl of 5 × SDS-PAGE loading buffer were added to each tube and the tubes were heated at 100°C for 5 min. The samples were separated on a 6% SDS-PAGE and transferred onto PVDF membranes. The membranes were washed twice with TBST (150 mM NaCl, 2 mM KCl, 25 mM Tris, and 0.05% Tween-20, pH 7.4) and blocked for 1–1.5 h at room temperature with TBST containing 3% BSA. We then incubated the membranes at 4°C overnight with mouse monoclonal anti-CaSR (Abcam, ab19347; 1:500 diluted) or mouse anti-β-tubulin (Merck Millipore, Darmstadt, Germany, 05-661; 1:1000 diluted) contained in TBST with 3% BSA. Then, the membranes were washed thrice with TBST (5 min each) and incubated for 1 h at 37°C with goat anti-mouse IgG AP-conjugated (CWBIO, cw0111, or cw0110) diluted to 1:1000 in TBST with 3% BSA. After washing the membranes thrice with TBST, they were analyzed using a BCIP/NBT alkaline phosphatase color development kit (Beyotime Institute of Biotechnology, Shanghai, China). The relative quantities of proteins were determined using Image J software to analyze the sum density of each protein band image. The values of freshly ovulated oocytes were set to one and the other values were expressed relative to one. β-tubulin was used as the internal control.

Data analysis

Each treatment was repeated at least thrice. We first arcsine-transformed the percentage data and then analyzed them using ANOVA. Duncan's multiple comparison test was performed to identify differences. We used software (Statistics Package for Social Sciences) to conduct all data analyses. In this paper, all the data are expressed as mean ± standard error (SE) and statistical significance was set at $P < 0.05$.

Results

The STAS in oocytes recovered at different time points after hCG injection

Oocytes recovered 13, 19, and 25 h after hCG injection were treated with ethanol immediately after collection. The results showed that, although none of the newly ovulated oocytes were activated, 40% and 94% of the oocytes recovered 19 and 25 h after hCG injection were activated, respectively, following ethanol treatment (Fig. 1).

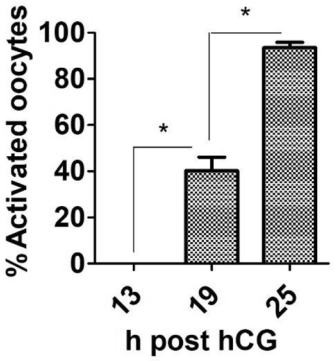

Fig. 1. Ethanol-activated percentages of oocytes recovered at different time points after hCG injection. The oocytes recovered at 13, 19, and 25 h after hCG injection were treated with ethanol immediately following collection. Each treatment was performed in triplicate with each replicate including approximately 30 oocytes. * Significant difference ($P < 0.05$) between treatments.

Effects of regulating CaSR activity on STAS and cytoplasmic calcium levels of newly-ovulated and aged mouse oocytes

Newly ovulated mouse oocytes recovered 13 h post-hCG were treated for 6 h with different concentrations of cinacalcet, an allosteric activator of the CaSR, and aged mouse oocytes recovered 19 h after hCG injection were treated for 6 h with different concentrations of the CaSR inhibitor NPS-2143 before examination for STAS and cytoplasmic calcium levels. The concentrations of cinacalcet and NPS-2143 tested in this study were as reported previously [24]. Activation rates of 13-h oocytes increased significantly with increasing cinacalcet concentrations and reached maximum by 0.5 μM of cinacalcet (Fig. 2A). In contrast, the activation rates of 19 h oocytes decreased significantly with increasing concentrations of NPS-2143 and reached minimum by 2 μM (Fig. 2B). Similarly, treatment with cinacalcet or NPS-2143 significantly increased and decreased the levels of cytoplasmic calcium in 13 h (Figs. 2C, E, and F) and 19 h oocytes (Figs. 2D, G, and H), respectively. Thus, activating CaSR increased STAS and cytoplasmic calcium in newly-ovulated mouse oocytes, whereas inhibiting CaSR decreased those in aged mouse oocytes, suggesting that CaSR is involved in the regulation of STAS of mouse oocytes.

Levels of CaSR in mouse oocytes recovered at different time points after hCG injection

Mouse oocytes recovered at 13, 19, 25, and 36 h post hCG injection were examined for CaSR levels using immunofluorescence microscopy and western blotting. CaSR was located both at the plasma membrane and within the cytoplasm in oocytes collected up to 25 h post-hCG injection, but the CaSR density, particularly in the cytoplasm, decreased significantly by 36 h after hCG injection (Figs. 3A–D). Western blotting showed one band at approximately 100–110 kDa representing the non-glycosylated form, two bands of approximately 120 and 140 kDa, which corresponded to the immature and mature glycosylated forms, respectively, and one band at 170–180 kDa representative of the dimeric active form of the CaSR (Fig. 3E). It should be noted that the dimeric and non-glycosylated forms of CaSR contained double thin bands. Although immunofluorescence quantification showed that the level of total CaSR protein did not change significantly up to 25 h after hCG injection (Fig. 3F), western blotting revealed significant increases in the functional dimer CaSR protein at 19 and 25 h post hCG injection compared to that in oocytes recovered 13 h post hCG injection (Fig. 3G). At 36 h post-hCG injection, both the total and dimer CaSR proteins decreased significantly. As the 19 h and 25 h oocytes showed significantly higher STAS than the 13 h oocytes (Fig. 1), and treatment with cinacalcet and NPS-2143 significantly increased and decreased, respectively, the STAS and cytoplasmic calcium levels (Fig. 2), the results suggest that STAS in mouse oocytes is positively correlated with the level of the functional CaSR dimer protein.

Effects of inhibiting CaSR with NPS-2143 while activating NCX with NaCl on STAS and cytoplasmic calcium levels of mouse oocytes

When mouse oocytes recovered 19 h after hCG injection were cultured for 6 h in CZB with 2 μM NPS-2134 or 75 mM NaCl [17] or both, the activation rate (Fig. 4A) as well as the cytoplasmic calcium level (Fig. 4B, C, and D) were significantly lower with NPS-2134 or with both NPS-2134 and NaCl than with NaCl alone. This suggests that the CaSR-controlled calcium channel is more powerful than the NCX channel in regulating cytoplasmic calcium and STAS of aging oocytes. This suggests that the NCX of mouse oocytes does not possess the capacity to pump out all the calcium released by the activation of the CaSR-controlled channel, leading to increased cytoplasmic calcium and STAS upon activating stimulation.

Roles of T- and L-type calcium channels in controlling STAS of aging mouse oocytes

Mouse oocytes recovered 19 h post hCG injection were aged for 6 h in CZB containing different concentrations of calcium channel blockers before examination for ethanol activation. The concentrations of ML218 and Nifedipine used in this study were those previously reported [24]. Neither ML218 (T-type channel blocker) nor nifedipine (L-type channel blocker) decreased the activation rate of mouse oocytes (Fig. 5). These results suggest that T- and L-type calcium channels are inactive in aging mouse oocytes, which rely primarily on the CaSR for cytoplasmic calcium regulation.

Discussion

This study showed that CaSR was localized in both the plasma membrane and cytoplasm of mouse oocytes. Using immunofluorescence microscopy, Dell'Aquila *et al.* [21] observed CaSR expression in human GV, MI, and MII oocytes, and De Santis *et al.* [22] detected

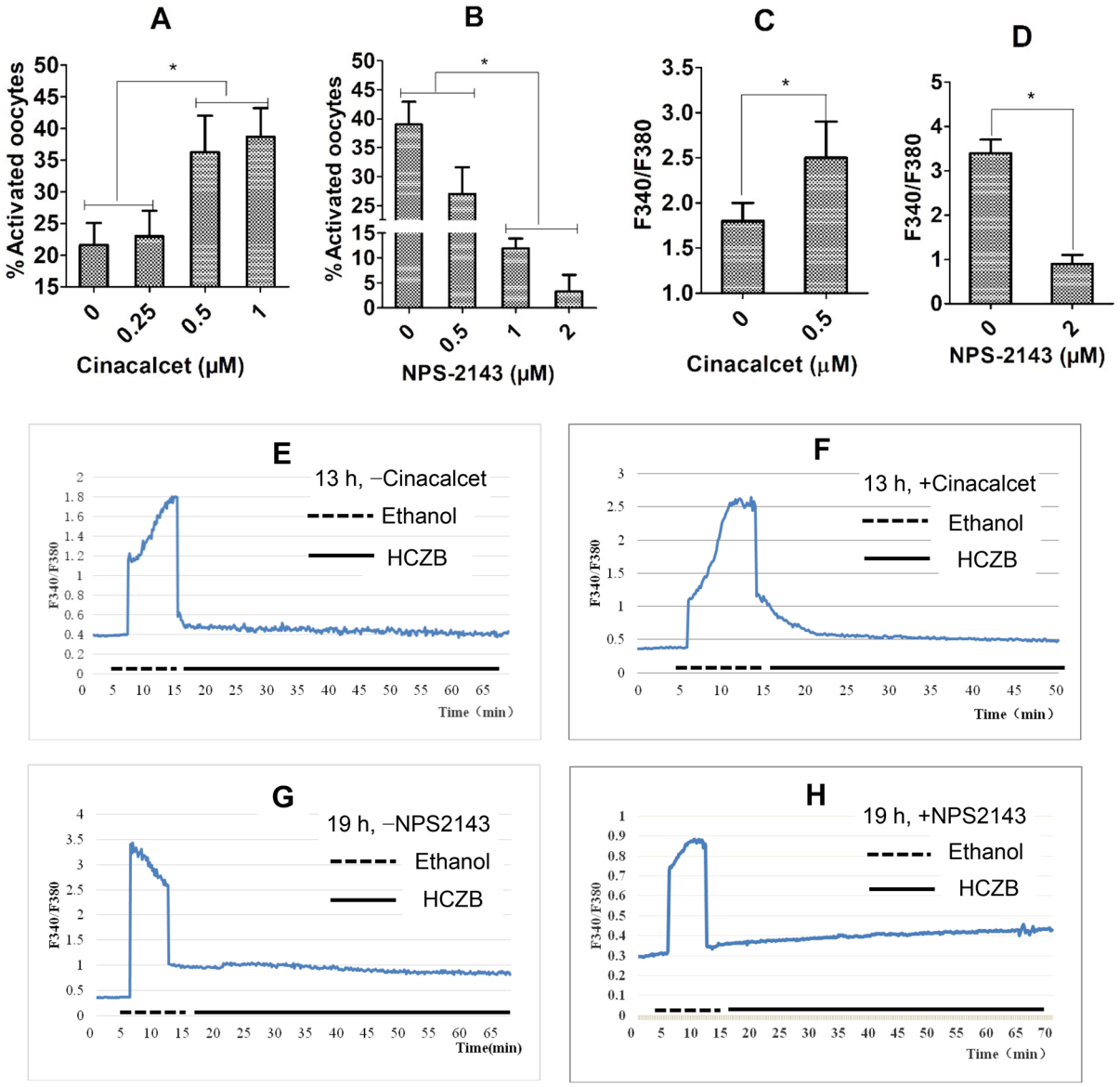

Fig. 2. Effects of activating or inhibiting CaSR on activation rates and cytoplasmic calcium levels of mouse oocytes. Newly ovulated oocytes recovered at 13 h after hCG were cultured for 6 h with various concentrations of cinacalcet to activate CaSR, and aged oocytes recovered at 19 h after hCG injection were cultured for 6 h with various concentrations of NPS-2143 to inhibit CaSR. At the end of the culture, oocytes were treated with ethanol for examination of STAS (graphs A and B) or assayed for levels of cytoplasmic calcium (graphs C and D). Panels E–H show dynamics of cytoplasmic calcium (F340/F380) in oocytes cultured without (−) or with (+) 0.5 μM cinacalcet (E and F) or 2 μM NPS-2143 (G and H). Oocytes were first monitored in HCZB medium for 5 min to record the baseline F340/380 ratio, then monitored for 10 min in HCZB containing 5% ethanol (dotted line), and finally, they were monitored in HCZB alone for 40 min (solid line). For STAS assessment, each treatment was performed in triplicate with each replicate including approximately 30 oocytes. For calcium measurement, each treatment was repeated in triplicate and each replicate included 20–30 oocytes. * Significant difference ($P < 0.05$) between treatments.

CaSR in the plasma membrane and, more pronounced, within the cytoplasm of horse oocytes at the GV, MI, and MII stages of meiosis. Western blotting revealed four bands at 100–110, 120, 140, and 170–180 kDa in mouse oocytes, representing the non-glycosylated, immature, mature glycosylated, and dimeric forms of the CaSR, respectively. Similar results have been reported for human vascular smooth muscle cells [26]. Using western blot analysis, Dell'Aquila *et al*. [21] and De Santis *et al*. [22] detected a single CaSR protein of 130 kDa in human and horse MII oocytes, respectively, and Liu *et al*. [23] detected a 160 kDa CaSR protein in porcine oocytes. In addition, we observed double thin bands for the dimeric and non-glycosylated forms of CaSR in mouse oocytes. CaSR doublets of 130/120 kDa were observed in human [21] and equine [22] cumulus cells.

Both previous studies [9, 17, 27] and the present study demonstrate that aged oocytes are more susceptible to activation stimuli than newly ovulated oocytes. Thus, this study showed that, although none of the newly ovulated mouse oocytes recovered 13 h after hCG injection was activated, 40% and 94% of the oocytes recovered 19 and 25 h after hCG injection were activated, respectively, following ethanol treatment. Although immunofluorescence indicated that the density of total CaSR protein in mouse oocytes remained constant up to 25 h post hCG injection, western blotting showed that the functional dimer protein of CaSR increased significantly from 13 h to 25 h after hCG injection. This suggests that STAS in aging oocytes is positively

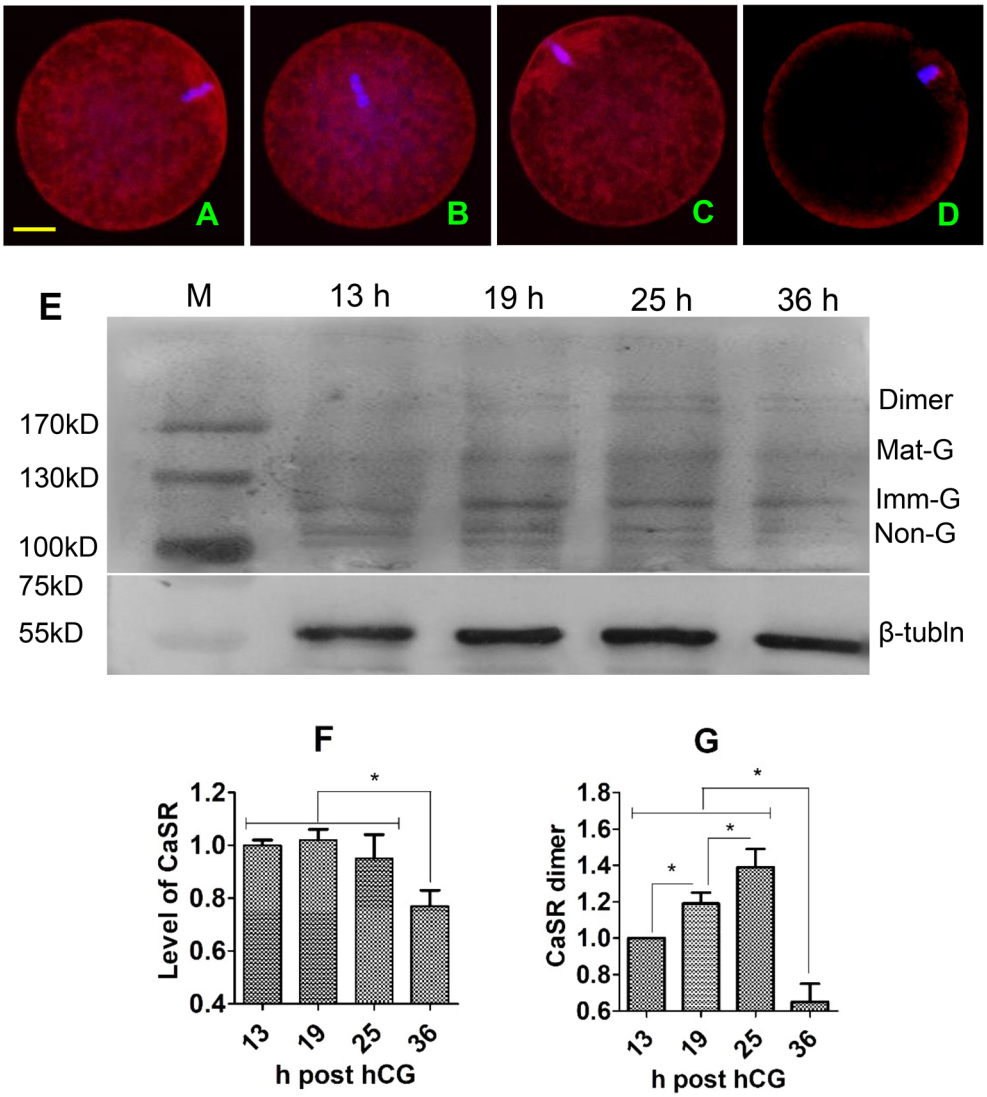

Fig. 3. Levels of CaSR in mouse oocytes recovered at different time points after hCG injection. A, B, C, and D show oocytes recovered at 13, 19, 25, and 36 h post hCG injection, respectively. These micrographs are merged confocal images with DNA and CaSR protein colored blue and red, respectively. Scale bar: 15 µm. Panel E shows full blots of western blotting displaying bands of dimmer, mature glycosylated (Mat-G), immature glycosylated (Imm-G), and non-glycosylated (Non-G) CaSR proteins. Graph F shows quantification of total proteins of CaSR by immunofluorescence. Each treatment was performed in triplicate with each replicate including approximately 30 oocytes. Graph G shows quantification of CaSR dimmer by western blotting. Each treatment was performed in triplicate and each replicate included 200 oocytes. * Significant difference ($P < 0.05$).

correlated with the level of the functional dimer protein of CaSR, but not with the level of its total protein, and that the activation of CaSR by non-specific or specific stimuli facilitates the dimerization of CaSR monomers during oocyte aging.

Previous studies have shown that the CaSR on the cell plasma membrane functions as a homodimer to sense minute alterations in extracellular calcium levels [28, 29]. For instance, Pidasheva *et al.* [29] suggested that constitutive dimerization of the CaSR occurs in the endoplasmic reticulum, which is necessary for the exit of the receptor from the endoplasmic reticulum and trafficking to the cell surface. Summarizing studies using natural CaSR mutations, Bai [30] highlighted the functional importance of CaSR dimerization. Furthermore, it has been reported that the activation of the CaSR promotes its expression, trafficking, and membrane insertion. For instance, agonist-driven maturation and plasma membrane insertion of the CaSR dynamically control signal amplitude [31].

The results showed that the total and functional dimer proteins of the CaSR decreased dramatically from 25 to 36 h after hCG injection. The mechanisms underlying this dramatic decline in CaSR expression during the late stages of postovulatory aging require further investigation. Our explanation is that the CaSR protein in oocytes is degraded along with other proteins due to degeneration and apoptosis during the extended aging process. It has been reported that extended aging of oocytes causes damage to their components, such as deterioration of cytoskeletal components [32, 33] and initiation of apoptosis [34, 35].

Free calcium ions from both the extracellular medium and intracellular calcium stores can enter the ooplasm. The current results demonstrated that treatment with T- or L-type calcium channel blockers had no effect on the STAS of mouse oocytes recovered 19 h post-hCG, suggesting that L- and T-channels are inactive in aging oocytes. Furthermore, our results showed that when mouse oocytes recovered 19 h after hCG was treated with NPS-2134 to inhibit CaSR or with NaCl to activate NCX, both the activation rate and the

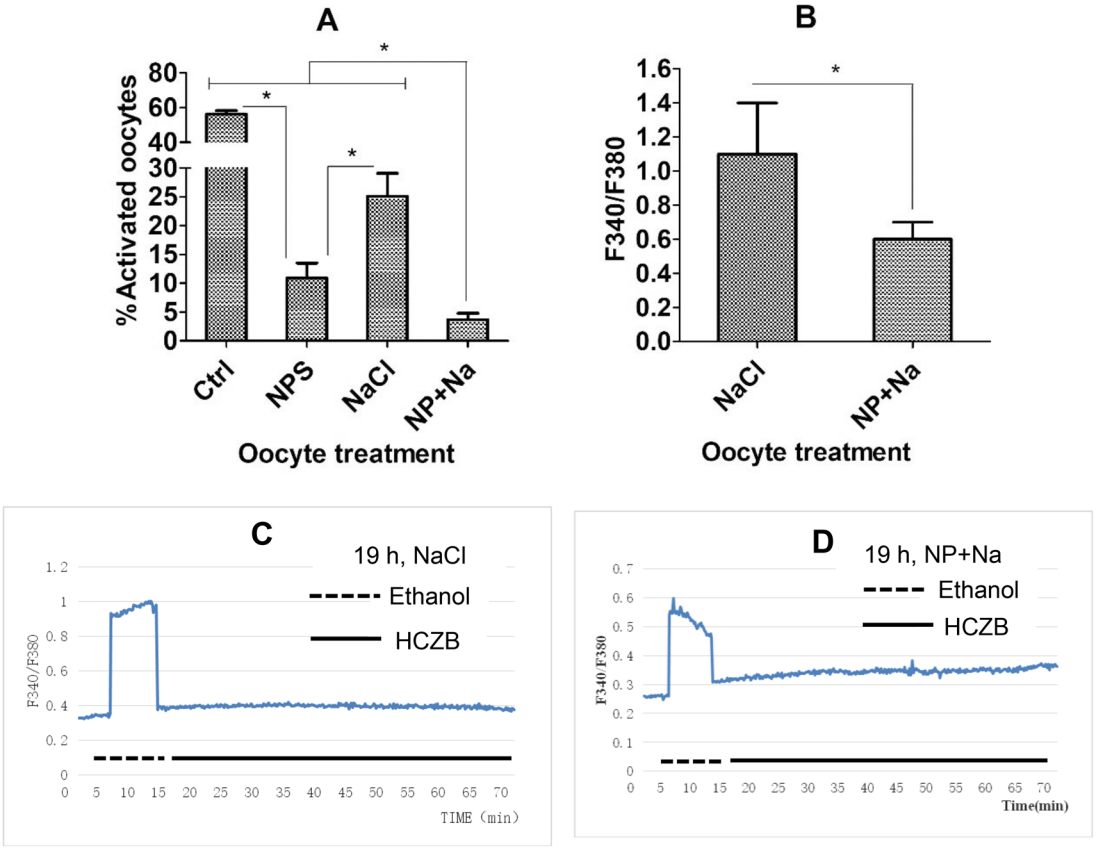

Fig. 4. Effects of inhibiting CaSR with NPS-2143 and activating NCX with NaCl on STAS and cytoplasmic calcium levels of mouse oocytes. Mouse oocytes recovered 19 h after hCG injection were cultured for 6 h in CZB containing 2 μM NPS-2134 (NPS) or 75 mM NaCl or both (NP + Na) before activation treatment or calcium measurement. Control (Ctrl) oocytes were cultured for 6 h in CZB with neither NPS-2134 nor NaCl. Graph A shows percentages of ethanol-activated oocytes and graph B shows levels of cytoplasmic calcium (F340/F380) after different treatments. Each treatment was performed in triplicate with each replicate including 20–30 oocytes. * Significant difference ($P < 0.05$) between treatments. Panels C and D show F340/F380 plots in NaCl and NP + Na treated oocytes, respectively. Oocytes were first monitored in HCZB medium for 5 min to record the baseline F340/380 ratio, and then, monitored for 10 min in HCZB containing 5% ethanol (dotted line), and finally, they were monitored in HCZB alone for 40 min (solid line).

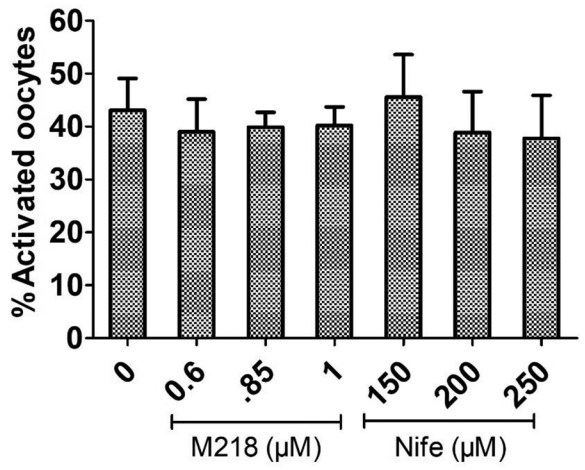

Fig. 5. Effects of treatment with calcium channel blockers on rates of ethanol-activation of mouse oocytes. Mouse oocytes recovered at 19 h post hCG injection were cultured for 6 h with different concentrations of calcium channel blockers, ML218 (M218) or Nifedipine (Nife). Each treatment was performed in triplicate with each replicate including approximately 30 oocytes. No significant difference was observed between treatments ($P > 0.05$).

cytoplasmic calcium level were significantly lower with NPS-2134 or with both NPS-2134 and NaCl than with NaCl alone, suggesting that the CaSR-controlled calcium channel is more powerful than the NCX channel in regulating cytoplasmic calcium and STAS in POA oocytes. Thus, the present results provide new evidence that the CaSR plays a more important role than other calcium channels in the regulation of STAS expression in POA mouse oocytes. This is different from aging rat oocytes, where multiple calcium channels are readily activated by nonspecific stimuli, leading to spontaneous activation [24, 36]. However, it remains unclear whether Ca^{2+}-ATPase, another mechanism that extrudes Ca^{2+} across the plasma membrane [37], plays a role in regulating STAS in POA mouse oocytes. Although NCX requires a high threshold level, Ca^{2+}-ATPase requires a low threshold level of cytoplasmic Ca^{2+} to activate [38].

In summary, we studied the CaSR expression in mouse oocytes and its role in regulating STAS in POA oocytes. The results indicated that CaSR is expressed in ovulated mouse oocytes, and it is involved in the regulation of STAS in POA oocytes, and plays a more important role than the other calcium channels tested in this connection. This is an important report on the expression of the CaSR in mouse oocytes and its role in the regulation of oocyte aging. These data are important for understanding the mechanisms of not only oocyte POA, but also STAS in aging oocytes.

Conflict of interests: The authors have nothing to declare.

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