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Regulation of LH Receptor and PGF2α Receptor Signaling by the Regulator of G Protein Signaling 2 (RGS2) in Human and Mouse Granulosa Cells

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Abstract

Regulators of G protein signaling (RGS) proteins bind the G protein Gα subunit in its active GTP-bound state and accelerate its GTPase activity, thus halting Gα activity. Induction of RGS2 expression has been previously shown in the rat ovary in response to ovulatory stimulation; however, the significance of RGS2 in the ovary has not been established. This study reports the potential role of RGS2 in the signaling of two G protein-coupled receptors, the LH and PGF2α (FP) receptors, in the human and the mouse granulosa cell lines, KGN and NT-1. The RGS2 mRNA concentration was rapidly and transiently elevated by human chorionic gonadotropin (hCG) or PGF2α analogue cloprostenol and this was followed by a decline to basal level at 24 h. Expression of the downstream critical target gene of the LH and FP receptor signaling pathways, namely, cyclooxygenase 2 (COX2), was induced by hCG but was inhibited by cloprostenol. Overexpression of RGS2 attenuated hCG-induced COX2 transcription. However, this augmented cloprostenol-mediated suppression of COX2 transcription. Confocal microscopy and immunoblot analysis were adopted to monitor the intracellular localization of RGS2 in COS-7 cells carrying the FP receptor and expressing RGS2-GFP or FLAG-RGS2. RGS2 was initially located predominantly in the nucleus and activation of the FP receptor resulted in RGS2 translocation from nucleus to the cell membrane. Thus, RGS2 expression was upregulated by LH receptor and FP receptor activation and modulation of partner receptor signaling by RGS2 may require RGS2 translocation from the nucleus to the plasma membrane.

Key Words: G protein, RGS, granulosa cell, gene regulation

Introduction

Ovulation and luteal regression are two key events that regulate female reproductive cycling. Mammalian ovulation is initiated by the coupling of a gonadotropin hormone such as luteinization hormone

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(LH) to a G protein-coupled receptor (GPCR) LH receptor (34). Activation of the LH receptor stimulates adenylyl cyclase in the ovarian granulosa cells and results in an elevation of the intracellular cAMP concentration. This then mediates activation of protein kinase A (PKA), phosphorylation of cAMP response element binding protein (CREB) and activation of transcription, which eventually leads to ovulation (13). On the other hand, luteal regression is also primarily triggered by activation of another GPCR, the prostaglandin F2α (FP) receptor. This results in activation of protein kinase C (PKC) leading to new gene transcription and luteolysis (19, 40). In both ovulation and luteolysis, prostaglandins appear to be critical and inducible cyclooxygenase-2 (COX2). This has been well recognized as an essential regulator of prostaglandin synthesis (43) which is involved in both ovulation and luteolysis (36, 51). Expression of COX2 has been demonstrated to be regulated by activation of the LH and FP receptors, indicating that the LH and FP receptor signaling pathways can regulate COX2 expression which leads to prostaglandin production and harmonization of ovarian cycling (36, 51).

As intermediaries between the cell surface receptors and the intracellular effector systems, G proteins play a crucial role in determining the intensity and specificity of hormone signals. Upon agonist binding to a cognate receptor, the G protein Gα subunit transits from a GDP-bound to a GTP-bound state and liberates the G protein Gβγ subunit. Depending on the system, either Gα or Gβγ or both subunits can then activate downstream effectors. Once the GTP is hydrolyzed, the subunits reassociate and the signal is stopped (31). The intensity of the signal depends on the rates of GTP binding and hydrolysis, events that are catalyzed by agonist-occupied receptors and regulator of G protein signaling (RGS) proteins, respectively (16, 27). One feature of the signal response systems in GPCRs is that prolonged stimulation leads to deactivation/desensitization (5, 42). RGS proteins comprise a family made up of more than 30 known members that have been implicated as negative regulators of heterotrimeric G protein signaling (25). Biochemical studies have suggested that RGS proteins may interact with G proteins, GPCRs, effectors and auxiliary molecules (1). RGS proteins have been demonstrated to bind the Gα subunits of the Gαi and Gαq families *in vitro* and dramatically enhance their intrinsic GTPase activity. This inactivates the functioning of that specific Gα subunit (25).

It has been previously shown that expression of RGS2 can be detected in rat mature ovarian granulosa cells in response to ovulatory stimulation (48). However, the roles of RGS2 in the ovarian system remain unclear. To characterize the significance of RGS2 in the regulation of LH receptor-mediated and FP receptor-mediated downstream target gene expression of COX2, we investigated the potential activities of RGS2 protein in two ovarian granulosa cell lines, KGN and NT-1, We found that COX2 transcription was regulated by LH and FP receptor activation and such regulation was modulated by RGS2. We also found that after receptor activation, RGS2 would translocate from the nucleus to the plasma membrane. Overall, the present findings suggest a mechanism whereby, in response to hCG and cloprostenol treatment in granulosa cells, RGS2 expression is upregulated to modulate LH receptor-mediated and FP receptor-mediated signaling events and such action may require the translocation of RGS2 from nucleus to cell membrane.

Materials and Methods

Chemicals and Reagents

Fetal bovine serum (FBS) was from HyClone (Logan, UT, USA). Western blot chemiluminescence reagent and the luciferase assay kit were purchased from PerkinElmer (Shelton, CT, USA). Restriction enzymes were purchased from New England (Beverly, MA, USA). SuperFect transfection reagent and plasmid purification columns were from Qiagen (Valencia, CA, USA). Reverse transcriptase and T4 DNA ligase were from Promega (Madison, WI, USA). Pfu DNA polymerase was from Stratagene (La Jolla, CA, USA). The PGF2α analogue cloprostenol was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). The Western blot transfer membrane immobilon-P was from Millipore (Bedford, MA, USA). Unless otherwise specified, other chemicals and reagents used in these studies were purchased from Sigma (St. Louis, MO, USA).

Plasmid Construction

A plasmid expressing RGS2-EGFP protein was constructed by amplifying the RGS2 ORF from total RNA isolated from a human KGN granulosa cell line (29) by RT-PCR using two primers that introduced two different restriction enzyme sites (Kpn I-RGS2 UP: AAGGTACCATGCAAAGTGCTATGTTCTTGG, Bam HI-RGS2 DOWN:TAGGATCCGCTGTAG-CATGAGGCTCTGTGG). The PCR product was subcloned into the vector pEGFP-N1 (Clontech; Mountain View, CA, USA) using the Kpn I and Bam HI sites. The sequence of cloned RGS2 ORF was confirmed by sequence analysis.

Cell Culture

The human ovarian granulosa cell line KGN

Table 1. Primer sequences and sizes of polymerase chain reaction products

Gene		Primer Sequence	PCR product (bp)
GAPDH	Sense	5'-TGT TCC AGT ATG ACT CCA CTC-3'	841
	Antisense	5'-TCC ACC ACC CTG TTG CTG TA-3'	
β-actin	Sense	5'-GGC ACC ACA CCT TCT ACA AT-3'	834
	Antisense	5'-CGT CAT ACT CCT GCT TGC TG-3'	
RGS2	Sense	5'-GAC CCG TTT GAG CTA CTT CTT-3'	494
	Antisense	5'-CCG TGG TGA TCT GTG GCT TTT TAC-3'	
RGS4	Sense	5'-TCC AGG CAA CCA AAG AGG TGA A-3'	202
	Antisense	5'-TGC TTT TCT GCC CCA CAG CT-3'	
RGS5	Sense	5'-GCC CCA CTC ATG CCT GGA AA-3'	321
	Antisense	5'-AGC TTT GCC AAC TCA GCC AT-3'	

GAPDH: glyceraldehyde-3-phosphate dehydrogenase

was maintained in Dulbecco's modified Eagle medium: nutrient mixture F-12 (Ham) (1:1) (DMEM/F-12) with 10% fetal bovine serum (FBS), 2 g/l sodium bicarbonate, 100 U/ml penicillin, 100 μg/ml streptomycin, in an atmosphere of 5 % CO_2 at 37°C. The mouse granulosa cell line NT-1 (35) was maintained in Dulbecco's modified Eagle medium: nutrient mixture F-12 (Ham) (1:1) (DMEM/F-12) with 10% fetal bovine serum, 20 mM HEPES, 4.5 g/l glucose and 100 U/ml penicillin, 100 μg/ml streptomycin, in an atmosphere of 5 % CO_2 at 37°C. A SV40-transformed monkey kidney fibroblast COS-7 cell line was maintained in complete Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 50 U/ml penicillin, 50 μg/ml streptomycin and 2 mM L-glutamine.

Experiment I: mRNA Measurements

Overnight plated cells were washed three times with serum-free medium and treated with hCG (5 IU/ml) or the PGF2α analogue cloprostenol (10 μM) for the indicated times. Total RNA was prepared from treated cells using Tri-Reagent (Sigma) according to the manufacturer's instructions. The isolated RNA samples were resuspended in RNase-free diethypyrocarbonate (DEPC)-treated water and kept at -80°C. A two step semi-quantitative RT-PCR method was used to measure mRNA expression of the RGS proteins in the treated and non-treated cells. Total RNA (5 μg) was combined with 0.5 μg oligo-dT, 200 μM dNTPs and H_2O and then preheated at 65°C for 2 min to denature secondary structure. The mixture was cooled rapidly to 4°C and then 10 μl 5X RT buffer, 10 μM DTT and 200 U MMLV reverse transcriptase (Promega) were added to give a total volume of 50 μl. The RT mix was incubated at 42°C for 90 min and then stopped by heating to 95°C for 5 min. The synthesized cDNA samples were subjected to PCR assay with the program of 30 cycles of 95°C for 30 sec, 55°C for 30 sec, 72°C for 1 min, and then followed by one cycle of 72°C for 5 min. The cDNA yields were determined by the signals from the internal standard house-keeping genes β-actin or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) after amplification for 30 PCR cycles with appropriate setting parameters. The primer sequences used in this assay are listed in Table 1. The PCR products were subjected to electrophoresis on a 1.5% agarose gel with 1 μg/ml ethidium bromide. The DNA signal on the gel was captured and analyzed by ImageQuant 5.2 software (Molecular Dynamics; Boston, MA, USA).

Experiment II: Functional Analysis of RGS2

It has been previously shown that LH or FP receptor activation is able to regulate COX2 gene transcription in granulosa and large luteal cells, respectively (8, 46). Therefore, to evaluate the potential roles of the RGS protein in LH receptor- and FP receptor-mediated downstream signaling events, a COX2 promoter construct (52) was cotransfected with a control plasmid that expressed β-galactosidase and driven by the CMV promoter (CMV-β-GAL) in order to establish a monitoring system for LH receptor- and FP receptor-mediated COX2 transcription. Briefly, 1 μg of a COX2 promoter plasmid containing the full-length COX2 5'-flanking sequence, 0.1 μg of CMV-β-GAL internal control plasmid and 2 μl of the transfection reagent SuperFect were mixed to form a DNA-SuperFect complex. To achieve overexpression of the RGS2 protein, 0.1 μg of pcDNA3-RGS2 or empty pcDNA3 vector was included in the DNA-SuperFect complex. Before transfection, cells that were attached to 24-

well plates were washed three times with serum-free medium. The DNA-SuperFect mixture was added directly to cells. After 3 h incubation at 37°C, the cells were washed three times with serum-free medium and treated with appropriate chemicals. To measure luciferase activity in the cell lysates, the medium was removed and cells were washed once with 1X PBS and lysed with Glo luciferase lysis buffer (Promega). Cell lysates were centrifuged at 15,000 rpm for 5 s and the luciferase activity in the supernatant was measured using a commercial luciferase assay kit and monitored by a VICTOR2 multilabel counter (PerkinElmer). Transfection efficiency was determined by measuring the β-galactosidase activity within the same sample. In brief, the β-galactosidase activity was determined by adding 20 μl of a freshly prepared solution of 83 μM fluorescein di-β-D-galactopyranoside (Sigma), 137.5 mM PIPES (pH 7.2) with 2.5% Triton X-100 and incubating at 37°C until a bright yellow color appeared. The reaction was stopped by the addition of 20 μl of 1 M Na_2CO_3 and the fluorescence activity was monitored using excitation of 485-nm and emission of 530-nm. Luciferase activity of samples was normalized against the transfection efficiencies and expressed as a fold increase relative to the control treatment. In the RGS2 overexpression experiment, after the normalization of the luciferase activity to the β-galactosidase activity, the basal COX2 promoter activity without RGS2 overexpression (empty vector group) was defined as 1 to investigate the effects of RGS2 on basal and hCG- or cloprostenol-regulated COX2 promoter activity.

Experiment III: Localization of RGS2

Previously we have tried three different commercial RGS2 antibodies in immunoblotting assay in order to monitor endogenous RGS2 protein in KGN and NT-1 cells, and we found that all three antibodies recognized several different size proteins, rather the predicted 24 kDa RGS2 protein. By overexpressing FLAG-RGS2 in COS-7 cells, we confirmed that none of these antibodies recognized the FLAG-RGS2 protein. However, the FLAG-RGS2 fusion protein was recognized by the FLAG antibody. The lack of validated antibody prompted us to overexpress FLAG-RGS2 and RGS2-GFP to monitor intracellular localization of the RGS2 indirectly. Thus, to monitor the intracellular RGS2 localization before and after receptor activation, a well-known high transfection rate cell line, COS-7 cells were seeded onto gelatine-coated cover slides and cultured overnight to reach 80% confluence. Plasmid encoding an RGS2-EGFP fusion protein was transfected alone or in combination with a human FP receptor expression plasmid (33) using SuperFect reagent. After a 3-hr post transfection, the medium was replaced with fresh medium and cells were cultured overnight. The cells were then washed with 1X PBS and incubated with serum-free medium with or without 10 μM cloprostenol for 15 min. The cells were then fixed with 4% paraformaldehyde for 10 min, washed with 1X PBS and subject to confocal microscopy to monitor RGS2 localization. A second and different experimental approach was also used to examine the intracellular localization of RGS2. In this case, a plasmid overexpressing FLAG-RGS2 (4) and human FP receptor expression plasmid (33) were transfected into COS-7 cells with SuperFect and the cells were treated or not treated with cloprostenol (10 μM) for 2 h. The RGS2 protein in the nuclear fraction or in the total lysates was determined by immunoblot analyses using antibody against the FLAG tag.

Statistical Analyses

Results were analyzed by one-way ANOVA to test the effects of the treatments. In Experiment I, means were compared for different time points in the presence of hCG or cloprostenol relative to the 0 h point (control). In the experiment II, luciferase activities from different treatments were compared to the control group at the same time points. The luciferase activity in the RGS2 overexpressing group was further compared to the empty vector group with same control, the hCG or the cloprostenol treatment. In experiment III, the levels of nuclear or total FLAG-RGS2 from the cloprostenol treatment group was normalized using internal controls (histone and α-tubulin) and compared to the control treatment. Any significant differences are indicated by an asterisk.

Results

In both human and mouse granulosa cell lines, there was a significant induction of RGS2 mRNA expression by hCG (Fig. 1A and 1B). In human KGN cell line, RGS2 mRNA rapidly increased by 2 h, and it then returned to basal level by 24 h (Fig. 1A). Similarly, in mouse NT-1 cell line, there was a rapid increase up to 6 h and a return to basal level by 24 h (Fig. 1B). Similarly, RGS2 mRNA expression was also rapidly and transiently induced by cloprostenol by 2 h and returned to basal level by 24 h in both the human and mouse granulosa cell lines (Fig. 1, C and D). To clarify whether the induction by hCG or cloprostenol was selective for RGS2, we also examined the mRNA expression of RGS4 and RGS5, both of which belong to the R4 family of the RGS superfamily (25, 42). However, expression of RGS4 was not affected by hCG or cloprostenol treatment in the human or mouse granulosa cell lines (Fig. 2, A, B, C, and D). In addition, RGS5 mRNA expression was not

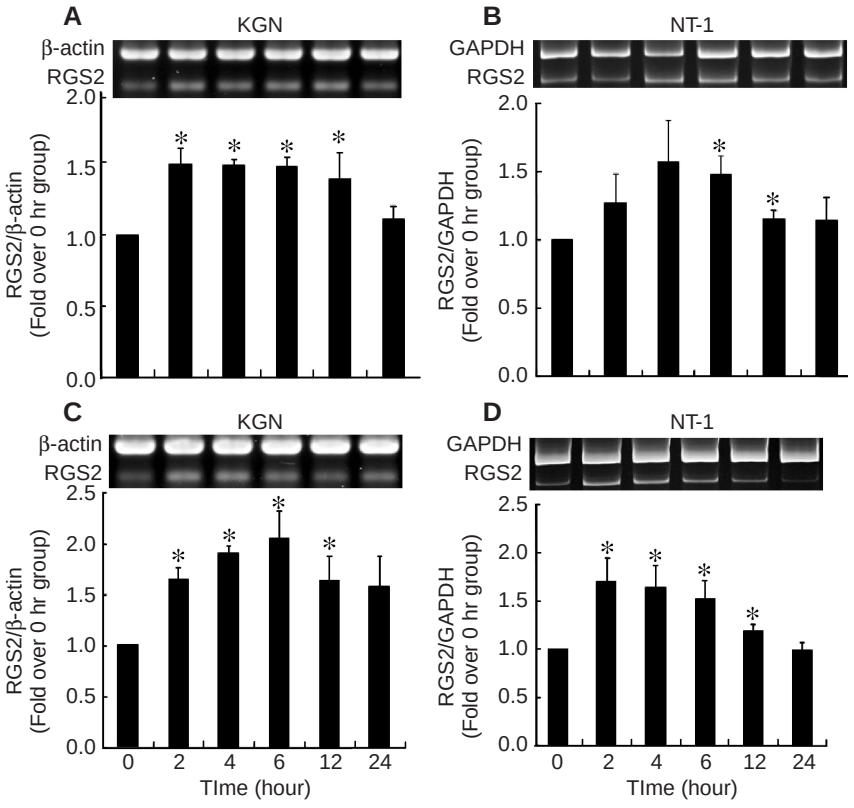

Fig. 1. hCG- and cloprostenol-induced RGS2 expression in granulosa cells. (A) Expression of RGS2 mRNA in cultured human granulosa cell line KGN treated with hCG (5 IU/ml) for the indicated times. (B) Expression of RGS2 mRNA in cultured mouse granulosa cell line NT-1 treated with hCG (5 IU/ml) for the indicated times. (C) Expression of RGS2 mRNA in cultured human granulosa cell line KGN treated with cloprostenol (10 μM) for the indicated times. (D) Expression of RGS2 mRNA in cultured mouse granulosa cell line NT-1 treated with cloprostenol (10 μM) for the indicated times. Results (ratio of RGS2/β-actin or RGS2/GAPDH) are shown as a fold increase over the control at 0 h. Significant differences ($P < 0.05$) between the time points and the control are indicated by an asterisk. The data represent the means ±SEM from 4 separate experiments.

affected by hCG or cloprostenol treatment in human granulosa cell lines (Fig. 2, E and F). No expression of RGS5 mRNA was detected in mouse NT-1 cells (data not shown).

In the NT-1 mouse granulosa cell line transfected with a full-length COX2 promoter construct (52), COX2 promoter activity was significantly induced by hCG at 24 h (Fig. 3A). However, cloprostenol treatment significantly suppressed COX2 promoter activity at 24 h (Fig. 3A). It was surprising that without any treatment, there was an increase of COX2 promoter activity in NT-1 cells (Fig. 3A). However, this finding was in agreement with a previous study demonstrating a spontaneous induction of COX2 promoter in primary cell cultures of ovine granulosa cells (53). When studying RGS2 function in hCG- and cloprostenol-mediated COX2 transcription, it was found that overexpression of RGS2 completely blocked hCG-induced COX2 promoter activity (Fig. 3B). Interestingly, overexpression of RGS2 did not blunt the suppression of COX2 promoter activity mediated by cloprostenol, but further augmented the inhibition effect of cloprostenol on COX2 promoter activity (Fig. 3B). Basal COX2 promoter activity was not affected by overexpression of RGS2 protein.

In the COS-7 cells transfected only with RGS2-GFP expressing plasmid, we found that GFP-tagged RGS2 localized predominantly within the nucleus (Fig. 4A), and that cloprostenol treatment did not cause any change to this pattern (Fig. 4A). To investigate the effects of receptor activation on RGS2 intracellular distribution, we coexpressed RGS2-GFP fusion protein together with human FP receptor (33). Coexpression of FP receptor without cloprostenol treatment resulted in a small level of membrane localization of RGS2-GFP (Fig. 4A). Nonetheless, cloprostenol treatment promoted a more dramatic increase in cell membrane localization of RGS2-GFP (Fig. 4A). To biochemically monitor the localization of RGS2 protein before and after FP receptor activation, COS-7 cells was transfected with plasmids expressing FLAG-RGS2 fusion protein and FP receptor. The amounts of FLAG-RGS2 protein in the

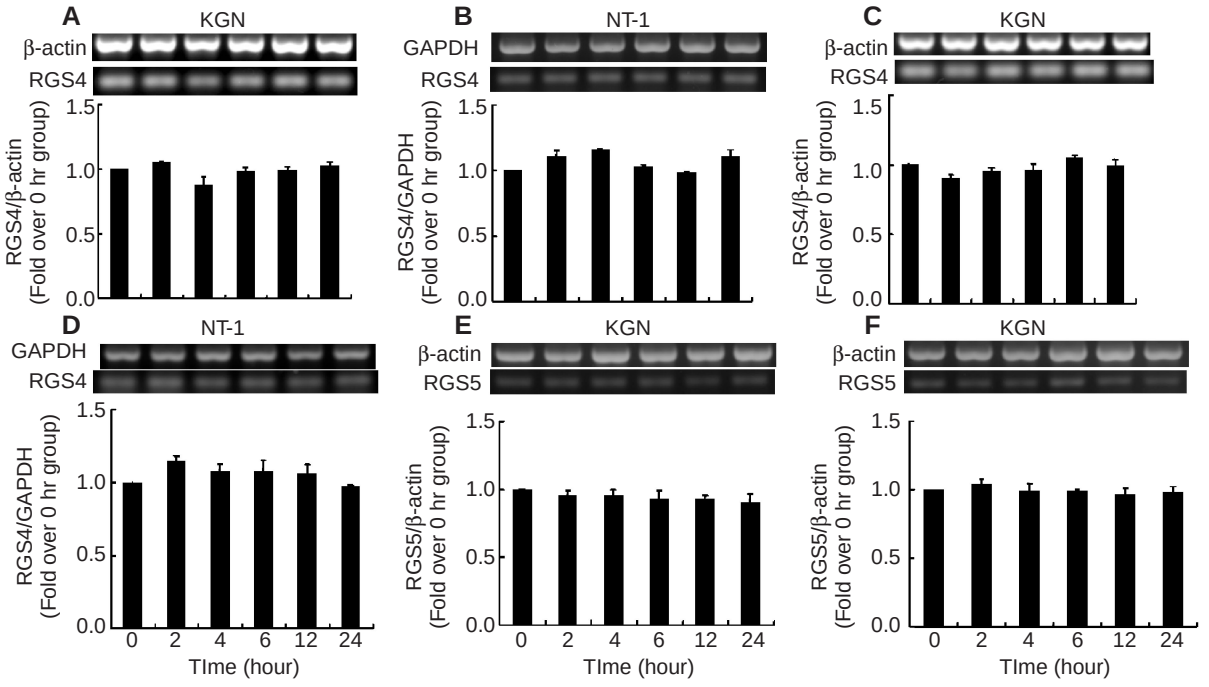

Fig. 2. RGS4 and RGS5 expression in hCG- and cloprostenol-treated granulosa cells. (A) Expression of RGS4 mRNA in cultured human granulosa cell line KGN treated with hCG (5 IU/ml) for the indicated times. (B) Expression of RGS4 mRNA in cultured mouse granulosa cell line NT-1 treated with hCG (5 IU/ml) for the indicated times. (C) Expression of RGS4 mRNA in cultured human granulosa cell line KGN treated with cloprostenol (10 μM) for the indicated times. (D) Expression of RGS4 mRNA in cultured mouse granulosa cell line NT-1 treated with cloprostenol (10 μM) for the indicated times. (E) Expression of RGS5 mRNA in cultured human granulosa cell line KGN treated with hCG (5 IU/ml) for the indicated times. (F) Expression of RGS5 mRNA in cultured human granulosa cell line KGN treated with cloprostenol (10 μM) for the indicated times. Results (ratio of RGS4/β-actin, RGS4/GAPDH or RGS5/β-actin) are shown as a fold increase over the control at 0 h. The data represent the mean ±SEM from 3 separate experiments.

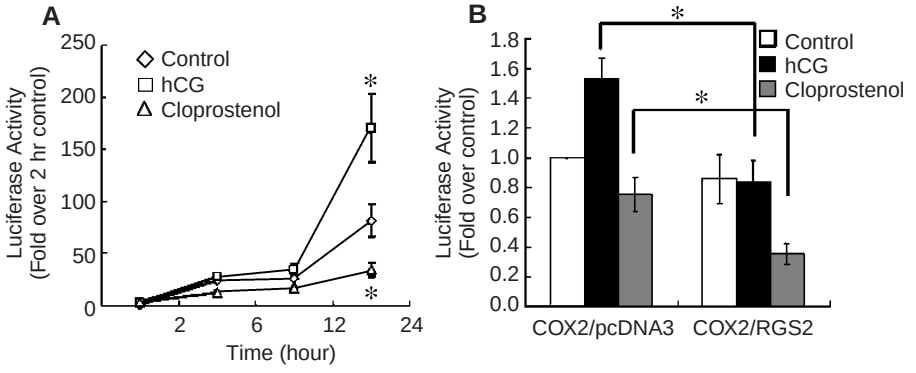

Fig. 3. Luciferase activity in lysates of mouse granulosa NT-1 cells transfected with COX2 promoter construct and a CMV-β-Gal control plasmid. (A) Cultured mouse NT-1 granulosa cells were transfected with the luciferase expression vector driven by a ovine COX2 promoter sequence (1500 bp) and a CMV-β-Gal control plasmid, treated with hCG (5 IU/ml) or cloprostenol (10 μM) for 2, 6, 12 or 24 h. (B) Cultured mouse NT-1 granulosa cells were transfected with the same plasmids as in (A) in combination with an empty pcDNA3 vector or pcDNA3-RGS2 plasmid and treated with hCG (5 IU/ml) or cloprostenol for 24 h. Luciferase activity data are normalized for the transfection efficiency (β-galactosidase activity) and represent the mean ±SEM for four (A) or three (B) different experiments, with each treatment evaluated in triplicate wells in each experiment. In (B), to examine the effect of RGS2 overexpression on the COX2 transcription, the control with pcDNA3 vector was defined as 1 and all the other groups were compare with it and expressed as fold over control. Significant differences ($P < 0.05$) between treatments and control at a given time point (A) or significant differences ($P < 0.05$) due to the RGS2 effect within the hCG or cloprostenol treatment group (B) are indicated by an asterisk.

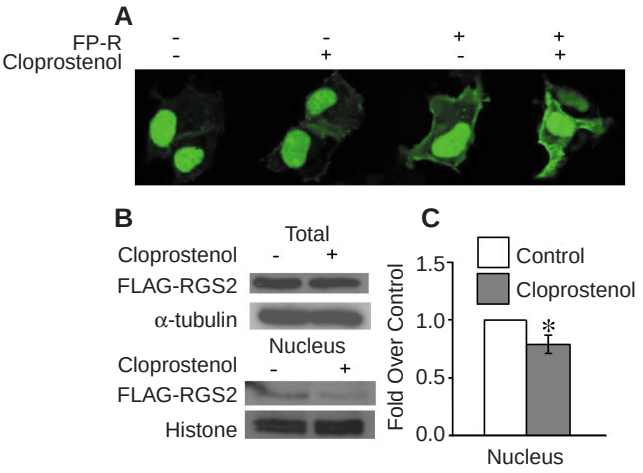

Fig. 4. Nuclear localization of RGS2 and receptor activation mediated translocation of nuclear RGS2. (A) Confocal images of COS-7 cells after transfection with pEGFP-RGS2 in combination with an empty vector or a human PGF2α receptor (FP-R) expressing plasmid. Overnight after transfection, the cells were treated or not treated with cloprostenol (10 μM) for 15 min. (B) COS-7 cells were transiently transfected with pFLAG-RGS2 plasmid and a human FP-R expressing plasmid and treated or not treated with cloprostenol (10 μM) for 2 h. Localization of FLAG-RGS2 was monitored by immunoblot analyses of total cell lysates and the nuclear fraction. (C) The quantities of FLAG-RGS2 in the nucleus were normalized using the internal control histone. The cloprostenol treatment was compared with a non-treatment group. The data represent the mean ± SEM from four separate experiments. Differences ($P < 0.05$) of cloprostenol treatment compared to the untreated group are indicated by an asterisk.

total cell lysates and in the nuclear fractions were also examined. The total amount of FLAG-RGS2 in the total lysates did not differ between the control and cloprostenol-treated cells (Fig. 4B). However, the amount of nuclear FLAG-RGS2 significantly decreased in the cloprostenol-treated cells (Fig. 4, B and C).

Discussion

In the ovarian system, the luteinizing hormone (LH) and prostaglandin F2α (FP) receptors are two critical G protein-coupled receptors (GPCRs) that regulate COX2 transcription as a downstream target player to maintain the fine-tuning of the reproductive cycle (2, 3, 34, 49). A previous study reported an induction of RGS2 prior to ovulation in rat ovarian granulosa cells (48). However the functional significance of RGS2 in the reproductive system has remained unknown. This project characterized the expression profile of RGS2 mediated by hCG and PGF2α in ovarian granulosa cells and primarily evaluated its potential function as part of hCG- and PGF2α-regulated downstream target gene expression. To our knowledge, this study is the first to demonstrate that RGS2 is involved in the LH receptor-regulated and FP receptor-regulated downstream signaling events such as COX2 gene transcription. We have also demonstrated that the intracellular localization of RGS2 before and after cognate receptor activation appears to be different.

One feature of GPCR signaling is the selective coupling of the receptor to G proteins and effectors, as well as selective interaction between the G proteins and the RGS proteins (1, 25). It has been demonstrated that the LH receptor couples with Gαs and this leads to PKA activation (34), and that activation of the LH receptor signaling pathway can be desensitized by downregulating its downstream target genes, for example, StAR expression (34). The interacting RGS partner of the LH receptor signaling was not clear until a recent study. This study described the testing of the effects of candidate RGS proteins on the LH receptor binding of its ligand and the coupling of its effector systems. RGS3 was able to inhibit LH receptor binding with the ligand (10). This article was the first to connect the negative regulator RGS protein with LH receptor signaling. However that the downstream signaling events after LH receptor binding by the ligand were not examined (10). Our results show that RGS2 was specifically upregulated by hCG treatment, and overexpression of RGS2 was able to block hCG-induced downstream target gene COX2 transcription. This indicates that RGS2 may act as a negative regulator of the LH receptor signaling in ovarian granulosa cells. The FP receptor has been shown to be expressed in granulosa cells (9, 15, 47), and its activation has been most linked to the G protein Gαq subtype (26, 33). This leads to PKC (26) or Rho activation (17) *via* Gαq activation. As the RGS partner to the FP receptor signaling route, it has been shown that RGS5 may inhibit the signal transduction initiated by FP receptor activation (33). However, in our results, expression of RGS2 but not that of RGS5 was induced by PGF2α analogue cloprostenol and overexpression of RGS2 further promoted an inhibition of the cloprostenol-mediated blockade of COX2 transcription. Therefore, LH receptor and FP receptor activation both induced RGS2 expression in granulosa cells. However, overexpressing RGS2 may regulate LH receptor- and FP receptor-mediated COX2 transcription in different ways. This suggests that RGS2 may modulate the LH receptor- and FP receptor-induced signaling pathways using different scenarios, not necessarily always by desensitizing its associated receptor signaling, since more and more novel functions of RGS proteins have been proposed as

more and more studies have been conducted (1, 22). How RGS2 differentially and substantially regulate LH receptor- and FP receptor-mediated COX2 expression needs further investigation.

Rapid and transient induction of RGS2 by cellular stimulation has been demonstrated in a variety of cell types (20, 28, 30, 45, 55). Our findings on RGS2 induction by hCG or cloprostenol in granulosa cells were similar to those of the studies. The functions of RGS2 in the various signaling pathways have also been documented in several studies. RGS2 has been shown to inhibit a Gαs-dependent increase in intracellular cAMP (38), to attenuate a Gαi-mediated inhibition of adenylyl cyclase activity (32), to downregulate Gαq-mediated phospholipase C activation (54), and to increase intracellular calcium (18). Our results suggest that RGS2 may interact with the Gαs and Gαq signaling pathway in ovarian granulosa cells because the overexpression of RGS2 affected the LH receptor-mediated and FP receptor-mediated signaling in terms of COX2 transcription. The reason for believing this is that in granulosa cell LH and FP receptors couple primarily with Gαs and Gαq, respectively (2, 3, 34). In addition, induction of RGS2 expression by a given signaling pathway may cross-regulate another signaling pathway, which has been shown to happen with RGS2 and the Gαs pathway. However, it can also inhibit the signaling activity from the Gαq pathway. Thus, a cross-desensitization relationship is formed between the Gαs and Gαq pathways (30, 39). Whether such a cross-inhibition mechanism by RGS2 occurs in granulosa cells needs further investigation.

RGS proteins have been demonstrated to differentially localize in the cytoplasmic, nuclear or Golgi compartments and the RGS2 has been shown to generally accumulate in the nucleus (11, 44, 54). The nuclear localization of RGS2 has been shown to be affected by overexpression of the G protein Gα subunit (12, 37, 38), GPCRs (12, 37, 38) and the effector system adenylyl cyclase (6, 37). It has been reported that the N-terminal sequence of RGS2 is required for RGS2 intranuclear and membrane localization (23), suggesting that signal-induced redistribution of RGS2 may regulate its cellular functioning (23). Our results also support the idea that RGS2 is primarily localized in the nucleus and on cognate receptor activation is translocated from the nucleus to the cell membrane. Therefore, it appears that RGS2 may need to translocate from nucleus to the plasma membrane to execute the protein's functions with various cellular interacting partners. In fact, there have been accumulating data supporting the hypothesis that RGS2 associates with signaling complexes by interacting with a receptor or Gα subunit (6, 24), adenylyl cyclases (6) and a downstream kinase (14). RGS2 has also been demonstrated to interact with molecules unrelated to G protein signaling (22). Although it is well recognized that the main function of classical RGS proteins is to desensitize G protein signaling in the context of the Gα subunit by promoting GTP hydrolysis (5, 7, 42), there is also evidence suggesting that RGS2 can regulate ion channels. For example, TRPV6-induced calcium increase independent of its GAP (GTPase Activating Protein) activity on Gαq has been identified (41). Furthermore, there is also accumulating evidence to suggest that RGS proteins may also play a number of novel roles when interacting with several other non-G protein molecules (21, 22, 50).

It is well recognized that G protein selectivity in terms of RGS interaction is a determinant of RGS functioning (22, 24). In this study, defining the selective interaction relationship between RGS2 and LH/FP receptors is only the starting point toward expanding our knowledge of how RGS2 plays its many roles in modulating LH receptor-regulated and FP receptor-regulated signaling in the ovarian system. In conclusion, this study provides clear evidence that hCG-regulated or PGF2α-regulated COX2 gene transcription in granulosa cells is modulated by RGS2. Nuclear localization of RGS2 was also found and activation of its cognate receptor was able to drive RGS2 translocation from the nucleus to the plasma membrane. This leaves open questions as to how RGS2 modulates LH and FP receptor-mediated signaling events and whether this occurs at the level of the receptors, at level of the G proteins, at the level of the effector systems or even further downstream. Finally, there remains how RGS2 translocates from the nucleus to the cell membrane. All of these points deserve further investigation.

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