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Oxytocin stimulation of RGS2 mRNA expression in cultured human myometrial cells

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Park, Eun Sung, Clement O. Echetebu, Solweig Soloff, and Melvyn S. Soloff. Oxytocin stimulation of RGS2 mRNA expression in cultured human myometrial cells. *Am J Physiol Endocrinol Metab* 282: E580–E584, 2002. First published December 18, 2001; 10.1152/ajpendo.00437.2001.—Regulators of G protein signaling (RGS proteins) interact with $G\alpha_q$ and $G\alpha_i$ and accelerate GTPase activity. These proteins have been characterized only within the past few years, so our understanding of their importance is still preliminary. We examined the effect of oxytocin on RGS2 mRNA expression to help determine the role of RGS proteins in oxytocin signaling in human myometrial cells in primary culture. Oxytocin increased RGS2 mRNA concentration maximally by 1 or 2 h in a dose-dependent and agonist-specific manner. RGS2 mRNA levels were also elevated by treatment with Ca^{2+} ionophore, phorbol ester, or forskolin. Oxytocin's effects were completely inhibited by an intracellular Ca^{2+} chelator and partially blocked by a protein kinase C inhibitor, indicating that intracellular Ca^{2+} concentration is the primary signal for oxytocin elevation of RGS2 mRNA levels. Use of pharmacological inhibitors indicated that part of oxytocin-stimulated RGS2 mRNA expression is mediated by G_i/tyrosine kinase activities. Although oxytocin does not stimulate increases in intracellular cAMP concentration, agents that elevate intracellular cAMP concentrations and cause myometrial relaxation may possibly cause heterologous desensitization to oxytocin via RGS2 expression. These results suggest that RGS2 may be important in regulating the myometrial response to oxytocin.

intracellular calcium; protein kinase C; G proteins; forskolin; adenosine 3′,5′-cyclic monophosphate; regulator of G protein

PROTEINS TERMED REGULATORS of G protein signaling (RGS proteins) are a diverse multiprotein family that interacts with activated Gα subunits to block signaling by G_i and/or G_q classes of G proteins (12). The RGS protein binding results in accelerated hydrolysis of Gα-bound GTP. As part of the general class of GTPase-activating proteins, RGS proteins may be important for turning off many G protein-mediated physiological responses, accounting for attenuated responses or, in the extreme, what appears to be uncoupling of G protein activity from liganded receptors. GTPase-activating proteins have been characterized only within the past few years, so our understanding of their importance is still rudimentary. Several RGS mRNAs are constitutively expressed at high levels (8, 22), suggesting that the corresponding proteins might be readily available for the acute desensitization of signaling. In contrast, RGS2 mRNA levels are typically low in resting cells but are upregulated for several hours after stimulation by various agents in different cell types (11, 13, 14, 19, 22, 26). RGS2 mRNA is presumably synthesized in response to an initial stimulus and then blocks subsequent hormone-signaling events.

Oxytocin (OT) is a G protein-coupled receptor agonist that stimulates uterine smooth muscle contraction. Human myometrial cells in culture express OT receptors, and, although these cells are not in an appropriate environment to contract, they possess signal pathways involved in the stimulation of contraction. Thus myosin light chain kinase activity can be activated in myometrial smooth muscle cells by elevation of intracellular Ca^{2+} concentrations (2). OT also stimulates an increase in prostacyclin synthesis by a G protein-sensitive (pertussis toxin-inhibitable) pathway in cultured human myometrial cells (20). RGS2 presumably is expressed in all cell types, but nothing is known of its function in uterine smooth muscle cells. We have shown in the present studies that OT stimulates increased expression of RGS2 mRNA in human myometrial cells in primary culture and elucidated the major signal pathways involved.

MATERIALS AND METHODS

Reagents. Reagents were obtained from the following sources: OT and OT antagonist [d(CH_2)$_5$,Tyr(Me)^2,Thr^4,Tyr-NH_2^{9}]ornithine vasotocin from Peninsula Laboratories (Belmont, CA); pertussis toxin, GF-109203X, 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid-AM (BAPTA-AM), genestein, wortmannin, and phorbol 12-myristate 13-acetate (PMA) from Biomol Research Laboratories (Plymouth Meeting, PA); FBS from Atlanta Biological (Atlanta, GA); MEM and cell culture reagents from GIBCO-BRL (Grand Island, NY). All other chemicals were obtained from Sigma (St. Louis, MO).

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Myometrial cell culture. The University of Texas Medical Branch Committee on Research Involving Human Subjects approved the use of human tissue. Myometrial samples were taken from women by caesarean section near the end of gestation, and cells were prepared as described previously (5). The cells were maintained in MEM containing 10% (vol/vol) FBS, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 IU/ml penicillin G, 100 μg/ml streptomycin sulfate, and 15 μg/ml amphotericin B at 37°C (95% humidity) in the presence of 5% CO_2. The cells, which were used at confluence between passages 3 and 10, were serum starved overnight (about 16 h) before treatment with OT or other agents.

Northern blot analysis. Total RNA was isolated from cultured human myometrium cells using TRIzol reagent (GIBCO-BRL Life Technologies, Rockville, MD). Samples of 20 μg of RNA were subjected to electrophoresis in 1% agarose-formaldehyde gels and transferred to nylon membranes for Northern blotting. The RGS2 probe was obtained by RT-PCR and cloned into pGEM-T Easy (Promega, Madison, WI). We identified selected clones by restriction enzyme digestion and DNA sequencing. Hybridizations were performed using random primed cDNA fragments of the entire coding regions of RGS2 gene labeled with [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham Pharmacia Biotech, Piscataway, NJ) in ExpressHyb hybrization solution (Clontech Laboratories, Palo Alto, CA) overnight at 68°C. The blots were rinsed for 10 min at room temperature with 1× saline-sodium citrate (SSC) and 0.1% SDS solution and then 45 min at 65°C with 0.2× SSC and 0.1% SDS solution. The blots were exposed to a Cyclone phosphor screen (Packard Instrument, Meriden, CT) for image analysis and to X-ray film at −80°C for 1–3 days. After the films were developed, the nylon membranes were stripped and reprobed with the human α-tubulin cDNA, which was synthesized by RT-PCR, cloned, and labeled by random priming as described for the RGS2 probe.

Analysis of data. Experiments were repeated on myometrial cells derived from three separate women. The results of the Northern blots were analyzed densitometrically and quantified using ImageQuant software (Packard Instrument). In cases where a percent change is reported, the values were obtained by expressing the RGS2 mRNA concentration relative to that of α-tubulin mRNA. Because of variability in the results between cells in primary culture from different patients, the data are presented as a representative Northern blot from a triplicate set. The changes in RGS2 expression within each treatment group were comparable in all cases.

RESULTS

OT specifically increases RGS2 mRNA expression. Treatment of human myometrial cells with 100 nM OT resulted in an increase in the amount of RGS2 mRNA by 30 min (Fig. 1). Maximal stimulation was apparent by 1–2 h (Fig. 1). An effect of OT at 2 h was seen with as little as 0.1 nM OT, and 1 nM OT gave a near-maximal response (Fig. 2*A*). The effects of OT were blocked by the OT antagonist (Fig. 2*B*).

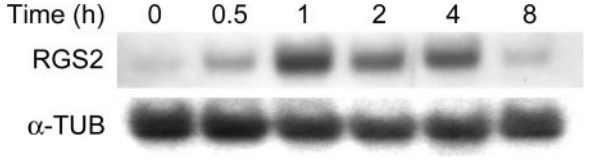

Fig. 1. Northern blot analysis of the effects of oxytocin (100 nM) on regulator of G protein (RGS2) mRNA levels in cultured human myometrial cells. After analysis, the blots were reprobed to determine α-tubulin mRNA concentration, which was used to normalize RGS2 mRNA levels. α-TUB, α-tubulin.

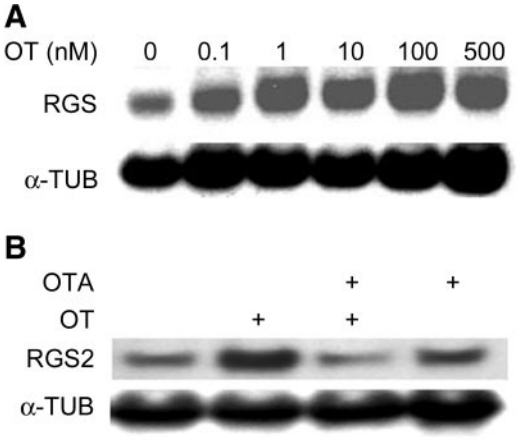

Fig. 2. Specificity of the RGS2 mRNA response to oxytocin (OT). *A*: dose-response relationship between OT concentration and RGS2 mRNA levels. Cells were treated with OT for 2 h. *B*: inhibition of the OT-stimulated RGS2 mRNA response by OT antagonist (OTA). Cells were treated either with 10 nM OT, 100 nM OT antagonist, or both for 2 h.

Mediators in OT-stimulated RGS2 mRNA expression. OT signaling in the myometrium has been shown to occur via G_q/phospholipase C (PLC) and activation of protein kinase C (PKC; see Refs. 9, 21, 24). Treatment of myometrial cells with the Ca^{2+} ionophore A-23187 (50 μM) to elevate intracellular Ca^{2+} concentrations resulted in an increase in RGS2 mRNA levels, with a maximal increase at ~2 h (Fig. 3*A*). A-23187 treatment, however, caused the degradation of mRNA at later time points, as was apparent by α-tubulin analysis (Fig. 3*A*). Increases in intracellular Ca^{2+} concentrations generated by OT treatment appear to be responsible for OT-stimulated expression of RGS2 mRNA, as pretreatment of cells with the Ca^{2+} chelator BAPTA-AM (10 and 30 μM) blocked the effects of OT (Fig. 3*B*). The effects of A-23187 treatment were also blocked by the two concentrations of BAPTA-AM (Fig. 3*B*).

OT signaling through stimulation of PLC activity results in activation of PKC. Activation of PKC by PMA also stimulated an increase in RGS2 mRNA 2 h after treatment (Fig. 4). This effect was blocked by pretreatment with the PKC inhibitor GF-109203X, at 1 and 10 μM. The effects of OT on RGS2 mRNA levels, however, were only partially blocked by the same doses of GF-109203X.

Increases in intracellular cAMP have been shown to induce increases in RGS2 mRNA levels in certain cell types but not others. Elevation of intracellular cAMP concentration by forskolin treatment caused a marked increase in RGS2 mRNA levels (Fig. 5). Thus human myometrial cells are in the category of cell type that responds to cAMP with an increase in RGS2 mRNA expression.

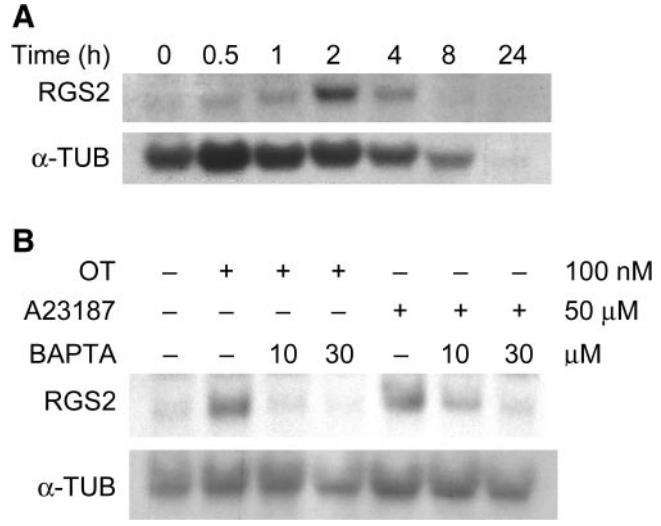

Fig. 3. Involvement of intracellular Ca^{2+} concentration on OT-stimulated RGS2 mRNA expression. *A*: effect of Ca^{2+} ionophore A-23187 after 2 h of treatment. *B*: effect of 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetraacetic acid-AM (BAPTA-AM) pretreatment on A-23187- and OT-stimulated RGS2 mRNA expression.

Synergy between signal pathway activators on RGS2 expression. The effects of A-23187 and PMA were potentiated by cotreatment with forskolin (Fig. 6). Likewise, A-23187 synergized with PMA to enhance RGS2 mRNA expression. The effects of PMA in conjunction with either A-23187 or forskolin were notable in that PMA alone was not stimulatory at the time point taken. The findings indicate that RGS2 mRNA levels are regulated along separate and complementary pathways with respect to increased intracellular Ca^{2+} concentrations, activation of PKC, and elevation of intracellular cAMP levels.

Other potential pathways mediating OT stimulation of RGS2 mRNA expression. The effects of OT on stimulation of inositol trisphosphate and increased intracellular Ca^{2+} concentrations are mediated by both pertussis toxin-sensitive and -insensitive pathways (25). Pretreatment of human myometrial cells with pertussis toxin (either 200 or 400 ng/ml) for 18 h blunted the increase in RGS2 expression stimulated by 2 h of treatment with OT (Fig. 7). There was a 32–51% reduction in OT-stimulated RGS2 expression with pertussis toxin treatment. These findings indicate that a portion of the effects of OT on RGS2 mRNA are mediated by $G_{i/o}$. Agonists for receptors coupled to $G_{i/o}$ have been shown to cross-talk through Gβγ to nonreceptor and receptor tyrosine kinase pathways (17). In keeping with the pertussis toxin effect, pretreatment of human myometrial cells with the tyrosine kinase inhibitor genestein (25 and 50 μM) for 2 h also partially inhibited (28–40%) the stimulation of RGS2 mRNA expression by 100 nM OT (Fig. 7).

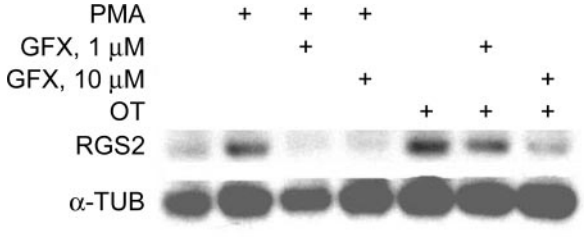

Fig. 4. Effect of protein kinase C (PKC) activation by phorbol 12-myristate 13-acetate (PMA; 100 nM) and inhibition of PKC activity by GF-109203X (1 and 10 μM) on RGS2 mRNA expression. Cells were pretreated with inhibitor for 30 min, followed by treatment with either PMA or OT (100 nM) for 2 h.

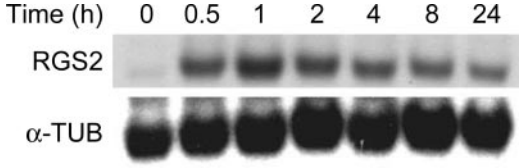

Fig. 5. Effect of forskolin (FSK; 25 μM) on RGS2 mRNA expression in human myometrial cells.

DISCUSSION

The RGS2 mRNA expression in human myometrial cells in primary culture was elevated in the presence of 10% FBS, but it was substantially diminished after 18 h of serum deprivation. Subsequent treatment of cells with OT caused a marked increase in RGS2 mRNA levels after 1 or 2 h, with a rise that was obvious after 30 min. Near-maximal effects were obtained with 1 nM OT, which is near the EC_{50} value for OT stimulation of intracellular Ca^{2+} and inositol phosphate elevation in human myometrial cells in primary culture (25). These findings indicate that the effects of OT on RGS2 mRNA are physiologically relevant. The effects of OT were ligand specific, since the OT antagonist blocked the rise in RGS2 mRNA levels.

There is still relatively little known about what regulates different RGS proteins. Based on the limited data available, the signal pathways involved in RGS2 mRNA expression appear to depend on cellular context. In human neuroblastoma SH-SY5Y cells, RGS2 mRNA levels are increased by activation of muscarinic receptors and a PKC-dependent mechanism (26). The RGS2 expression was not affected by increases in either intracellular Ca^{2+} or cAMP concentrations. PKC also appears to mediate RGS2 mRNA increases by ANG II in vascular smooth muscle cells (11). Tyrosine kinase inhibition and Ca^{2+} deprivation did not affect this increase in RGS2 mRNA concentration. A greater increase was seen in levels of RGS1 mRNA in human B

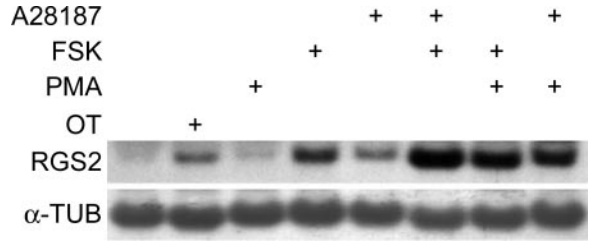

Fig. 6. Synergistic effects of A-23187 (50 μM), forskolin (25 μM), and PMA (100 nM) on RGS2 mRNA expression. Cells were treated for 2 h.

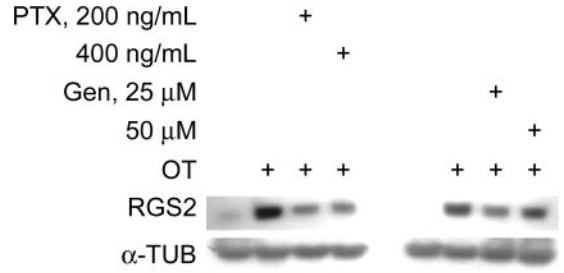

Fig. 7. Inhibition of OT-stimulated RGS2 mRNA expression by pertussis toxin (PTx) and genestein (Gen), a tyrosine kinase inhibitor. The myometrial cells were pretreated with either pertussis toxin for 18 h or with genestein for 30 min before treatment with OT for 2 h.

lymphocytes in response to a PKC activator than to a Ca^{2+} ionophore (ionomycin), whereas the opposite was true for RGS2 mRNA synthesis in blood mononuclear cells (13). Elevation of intracellular cAMP induces RGS2 expression in cultured rat osteoblasts (19) and PC-12 cells (22). Thus, depending on the cell type, RGS2 expression can be induced exclusively by PKC, intracellular Ca^{2+}, or cAMP. Each one of these pathways mediates the actions of G protein-coupled receptors and likely contributes to feedback regulation of the G proteins involved.

As we have shown in our present studies, there are multiple pathways that lead to increased RGS2 mRNA levels in human myometrial cells (Fig. 8). The primary signal that mediates the effects of OT appears to be linked to PLC-mediated stimulation of intracellular Ca^{2+} concentrations. OT also activates PKC via PLC-stimulated increases in diacylglycerol concentration. PKC likely catalyzes the phosphorylation of specific proteins that are involved in increased RGS2 mRNA levels. Partial inhibition of the effects of OT on RGS2 mRNA expression by pertussis toxin indicates that $G_{i/o}$ plays a role in signaling increases in RGS2 mRNA levels. In view of the similar degree of inhibition caused by pertussis toxin and genestein, we speculate that $G\beta\gamma$ linked to tyrosine kinase activation mediates a portion of the OT effect on RGS2 mRNA expression. This type of transactivation by $G\beta\gamma$ has been described in other G protein-coupled receptor systems (16, 17). Increases in intracellular cAMP concentrations also caused a rise in RGS2 mRNA levels, presumably independent of the OT receptor, which is not coupled to G_s. cAMP produces relaxation of smooth muscle by activation of cAMP-dependent protein kinase A, which interferes with several processes involved in smooth muscle contraction (3, 30). We speculate that there is the potential for heterologous regulation of OT action by agents that generate intracellular cAMP. The resulting increase in RGS2 expression might attenuate the responses to OT or other uterotonins acting via G proteins. Agents that increase myometrial cell cAMP levels include β-adrenergic drugs (15), relaxin (18), corticotropin-releasing hormone (10), calcitonin gene-related peptide (1), adrenomedullin (1), and parathyroid hormone-related peptide (29), among others.

The role of RGS2 in myometrial cell function remains to be established. It has been tacitly assumed that RGS2 functions as a GTPase. Thus an increase in RGS2 protein levels after an initial stimulation might be expected to attenuate subsequent signaling. In support of this possibility, Phaneuf and coworkers (23) found that exposing cultured human myometrial cells to OT for a prolonged period caused desensitization of the response to OT. There was a concomitant 90% reduction in the number of high-affinity receptor binding sites. Immunoblot and flow cytometry data indicated, however, that the total amount of OT receptor protein on the cell membrane surface was unaffected by OT treatment. These findings suggest that desensitization could occur at the G protein level (possibly involving RGS2) rather than at the receptor level.

Recent studies have indicated that RGS2 phosphorylation, along with RGS2 protein synthesis, may also be an important means of regulating GTPase activity. Phosphorylation of RGS2 by PKC in vitro diminished RGS2 activity stoichiometrically (28). There is evidence that RGS2 also has functions that are not associated with GTPase activity. RGS2 inhibits cAMP production by directly inhibiting the activity of adenylyl cyclase type III, the predominant adenylyl cyclase isoform in olfactory neurons (4). Although a clear understanding of the role in RGS2 mRNA in human myometrial cells remains to be established, the profound changes in RGS2 mRNA concentration elicited by OT suggest that RGS2 serves an important regulatory function with respect to OT action in these cells. Upregulation of RGS2 mRNA has also been demonstrated in vivo, in ovarian granulosa cells after human chorionic gonadotropin treatment (27). Because the myometrium undergoes marked changes in sensitivity to OT during pregnancy (6, 7), future studies are needed to

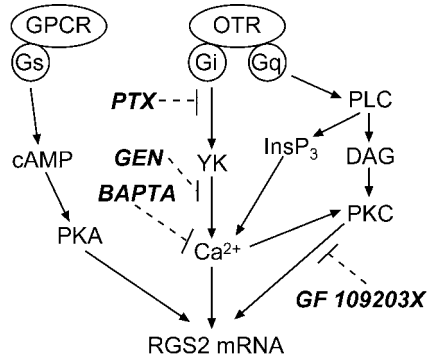

Fig. 8. Summary of multiple pathways involved in upregulating RGS2 mRNA expression in human myometrial cells in primary culture. The primary signal mediating the effects of OT was an elevation in intracellular Ca^{2+} concentration. PKC activation appeared to be secondary. Independent of the effects of OT, forskolin (which elevates intracellular cAMP concentrations) also increased RGS2 mRNA expression. About one-half of the OT effect is mediated by $G_{i/o}$ and tyrosine kinase activity, as reflected by inhibition by pertussis toxin and genestein, respectively. Tyrosine kinase activation has been shown in other systems to be coupled to G_i activation, presumably through $G\beta\gamma$ interactions. OTR, OT receptor; PLC, phospholipase C; DAG, diacylglycerol; PKA, protein kinase A; $InsP_3$, inositol trisphosphate.

elucidate the importance of RGS2 in the responsiveness of the myometrium to OT in vivo.

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REFERENCES

1. **Casey ML, Smith J, Alsabrook G, and MacDonald PC.** Activation of adenylyl cyclase in human myometrial smooth muscle cells by neuropeptides. *J Clin Endocrinol Metab* 82: 3087–3092, 1997.
2. **Chen C, Zheng B, Han J, and Lin SC.** Characterization of a novel mammalian RGS protein that binds to Gα proteins and inhibits pheromone signaling in yeast. *J Biol Chem* 272: 8679–8685, 1997.
3. **De Lanerolle P, Nishikawa M, Yost DA, and Adelstein RS.** Increased phosphorylation of myosin light chain kinase after an increase in cyclic AMP in intact smooth muscle. *Science* 223: 1415–1417, 1984.
4. **Dessauer CW, Srikumar D, Chen J, Yuen J, Yilma S, Dennis JC, Morrison EE, Vodyanoy V, and Kehrl JH.** RGS2 regulates signal transduction in olfactory neurons by attenuating activation of adenylyl cyclase III. *Nature* 409: 1051–1055, 2001.
5. **Echetebu CO, Ali M, Izban MG, Mackay L, and Garfield RE.** Localization of regulatory protein binding sites in the proximal region of human myometrial connexin 43 gene. *Mol Hum Reprod* 5: 757–766, 1999.
6. **Fuchs AR, Fuchs F, Husslein P, Soloff MS, and Fernstrom MJ.** Oxytocin receptors and human parturition: a dual role for oxytocin in the initiation of labor. *Science* 215: 1396–1398, 1982.
7. **Fuchs AR, Periyasamy S, Alexandrova M, and Soloff MS.** Correlation between oxytocin receptor concentration and responsiveness to oxytocin in pregnant rat myometrium: effects of ovarian steroids. *Endocrinology* 113: 742–749, 1983.
8. **Gold SJ, Ni YG, Dohlman HG, and Nestler EJ.** Regulators of G-protein signaling (RGS) proteins: region-specific expression of nine subtypes in rat brain. *J Neurosci* 17: 8024–8037, 1997.
9. **Grammatopoulos DK and Hillhouse EW.** Activation of protein kinase C by oxytocin inhibits the biological activity of the human myometrial corticotropin-releasing hormone receptor at term. *Endocrinology* 140: 585–594, 1999.
10. **Grammatopoulos DK and Hillhouse EW.** Role of corticotropin-releasing hormone in onset of labour. *Lancet* 354: 1546–1549, 1999.
11. **Grant SL, Lassegue B, Griendling KK, Ushio-Fukai M, Lyons PR, and Alexander RW.** Specific regulation of RGS2 messenger RNA by angiotensin II in cultured vascular smooth muscle cells. *Mol Pharmacol* 57: 460–467, 2000.
12. **Hepler JR.** Emerging roles for RGS proteins in cell signalling. *Trends Pharmacol Sci* 20: 376–382, 1999.
13. **Heximer SP, Cristillo AD, and Forsdyke DR.** Comparison of mRNA expression of two regulators of G-protein signaling, RGS1/BL34/1R20 and RGS2/G0S8, in cultured human blood mononuclear cells. *DNA Cell Biol* 16: 589–598, 1997.
14. **Ingi T, Krumins AM, Chidiac P, Brothers GM, Chung S, Snow BE, Barnes CA, Lanahan AA, Siderovski DP, Ross EM, Gilman AG, and Worley PF.** Dynamic regulation of RGS2 suggests a novel mechanism in G-protein signaling and neuronal plasticity. *J Neurosci* 18: 7178–7188, 1998.
15. **Lecrivain JL, Cohen-Tannoudji J, Robin MT, Coudouel N, Legrand C, and Maltier JP.** Molecular mechanisms of adenylyl cyclase desensitization in pregnant rat myometrium following in vivo administration of the β-adrenergic agonist, isoproterenol. *Biol Reprod* 59: 45–52, 1998.
16. **Leopoldt D, Hanck T, Exner T, Maier U, Wetzker R, and Nürnberg B.** Gβγ stimulates phosphoinositide 3-kinase-γ by direct interaction with two domains of the catalytic p110 subunit. *J Biol Chem* 273: 7024–7029, 1998.
17. **Luttrell LM, Della Rocca GJ, van Biesen T, Luttrell DK, and Lefkowitz RJ.** Gβγ subunits mediate Src-dependent phosphorylation of the epidermal growth factor receptor. A scaffold for G protein-coupled receptor-mediated Ras activation. *J Biol Chem* 272: 4637–4644, 1997.
18. **Meera P, Anwer K, Monga M, Oberti C, Stefani E, Toro L, and Sanborn BM.** Relaxin stimulates myometrial calcium-activated potassium channel activity via protein kinase A. *Am J Physiol Cell Physiol* 269: C312–C317, 1995.
19. **Miles RR, Sluka JP, Santerre RF, Hale LV, Bloem L, Boguslawski G, Thirunavukkarasu K, Hock JM, and Onyia JE.** Dynamic regulation of RGS2 in bone: potential new insights into parathyroid hormone signaling mechanisms. *Endocrinology* 141: 28–36, 2000.
20. **Molnar M, Rigo J Jr, Romero R, and Hertelendy F.** Oxytocin activates mitogen-activated protein kinase and up-regulates cyclooxygenase-2 and prostaglandin production in human myometrial cells. *Am J Obstet Gynecol* 181: 42–49, 1999.
21. **Morrison JJ, Dearn SR, Smith SK, and Ahmed A.** Activation of protein kinase C is required for oxytocin-induced contractility in human pregnant myometrium. *Hum Reprod* 11: 2285–2290, 1996.
22. **Pepperl DJ, Shah-Basu S, VanLeeuwen D, Granneman JG, and MacKenzie RG.** Regulation of RGS mRNAs by cAMP in PC12 cells. *Biochem Biophys Res Commun* 243: 52–55, 1998.
23. **Phaneuf S, Asboth G, Carrasco MP, Linares BR, Kimura T, Harris A, and Lopez Bernal A.** Desensitization of oxytocin receptors in human myometrium. *Hum Reprod* 4: 625–633, 1998.
24. **Phaneuf S, Carrasco MP, Europe-Finner GN, Hamilton CH, and Lopez Bernal A.** Multiple G proteins and phospholipase C isoforms in human myometrial cells: implication for oxytocin action. *J Clin Endocrinol Metab* 81: 2098–2103, 1996.
25. **Phaneuf S, Europe-Finner GN, Varney M, MacKenzie IZ, Watson SP, and Lopez Bernal A.** Oxytocin-stimulated phosphoinositide hydrolysis in human myometrial cells: involvement of pertussis toxin-sensitive and -insensitive G-proteins. *J Endocrinol* 136: 497–509, 1993.
26. **Song L, De Sarno P, and Jope RS.** Muscarinic receptor stimulation increases regulators of G-protein signaling 2 mRNA levels through a protein kinase C-dependent mechanism. *J Biol Chem* 274: 29689–29693, 1999.
27. **Ujioka T, Russell DL, Okamura H, Richards JS, and Espey LL.** Expression of regulator of G-protein signaling protein-2 gene in the rat ovary at the time of ovulation. *Biol Reprod* 63: 1513–1517, 2000.
28. **Waldo GL, Hollinger S, Hepler JR, and Harden TK.** Protein kinase C phosphorylates RGS2 and modulates its capacity for negative regulation of $G\alpha_{11}$ signaling. *J Biol Chem* 276: 5438–5444, 2001.
29. **Williams ED, Leaver DD, Danks JA, Moseley JM, and Martin TJ.** Effect of parathyroid hormone-related protein (PTHrP) on the contractility of the myometrium and localization of PTHrP in the uterus of pregnant rats. *J Reprod Fertil* 102: 209–214, 1994.
30. **Yue C, Dodge KL, Weber G, and Sanborn BM.** Phosphorylation of serine 1105 by protein kinase A inhibits phospholipase $C\beta_3$ stimulation by $G\alpha_q$. *J Biol Chem* 273: 18023–18027, 1998.