Expression and regulation of myometrial adenylyl cyclases (AC) were studied during pregnancy. Hybridization of poly(A)$^+$ RNA with specific cDNA probes for enzyme types I–IX indicated 1) the presence of transcripts encoding types II–VI and type IX in both species. No substantial change was observed in the amount of specific mRNA and basal AC activity from mid-pregnancy to term. However, activation of the $\alpha_2$-adrenergic receptor/Gi protein pathway resulted in potentiation of $G_s$-stimulated AC activity at mid-pregnancy but not at term (Mhaouty, S., Cohen-Tannoudji, J., Bouet-Alard, R., Limon-Boulez, I., Maltier, J. P., and Legrand, C. (1995) J. Biol. Chem. 270, 11012–11016). We demonstrate in the present work that $\beta y$ scavengers transducin-$\alpha$ and QEHA peptide abolished this positive input. On the other hand, increasing submicromolar concentrations of free Ca$^{2+}$, a situation that mimics late term, reduced the forskolin-stimulated AC activity with an $IC_{50}$ of 3.9 $\mu$M. Thus, the presence in myometrium of AC II family (types II, IV, VII) confers ability to G inhibitory proteins to stimulate enzyme activity via $\beta y$ complexes at mid-pregnancy, whereas expression of AC III, V, and VI isoforms confers to the myometrial AC system a high sensitivity to inhibition by Ca$^{2+}$-dependent processes at term. These data suggest that in the pregnant myometrium, the expression of different species of AC with distinct regulatory properties provides a mechanism for integrating positively or negatively the responses to various hormonal inputs existing either during pregnancy or in late term.

Data on hormonal regulation of myometrial contractility during the course of pregnancy implicate adenylyl cyclase (AC)$^3$ stimulatory pathways as a key component that may affect the degree of intracellular cAMP generation and consequently the contractile state of the uterus. Because one of the major sites of control of the biochemical events leading to uterine relaxation during normal pregnancy lies at the AC/cAMP system, the identification of AC isoforms in the pregnant myometrium is essential in understanding the influence exerted by the regulatory external signals (neurotransmitters and hormones) acting via G protein-coupled receptors. Hormonal control of AC activity is brought about by receptor-catalyzed activation of heterotrimeric G proteins that in turn regulate the cyclases by the release of $\alpha$G or $\beta y$ subunits or kinase activation. Recent studies have revealed an unexpected diversity of G protein-regulated AC by identifying nine distinct AC cDNA from various mammalian tissues (1–5). All of these isoforms of AC differ in their tissue distribution and their regulatory properties, providing a mode for different cells to respond diversely to similar external stimuli. Among all of the AC identified so far, the highly similar types II, IV, and VII form the largest known subfamily. Types II and IV share the property of being highly stimulated by $\beta y$ subunits of G$\alpha$ inhibitory proteins in the presence of activated G$\alpha_6$ (6, 7). These AC are also influenced by phosphorylation with protein kinase C (8–11). Types V and VI AC, a two-member subfamily, are inhibited directly by low levels of Ca$^{2+}$ (2, 12), whereas AC I and VIII are regulated positively by Ca$^{2+}$-calmodulin (13, 14). On the other hand, AC III can be phosphorylated by a calmodulin-dependent protein kinase II in response to the elevation of intracellular Ca$^{2+}$ which results, in vivo, in a 50% inhibition of hormone-stimulated enzyme (15). The novel ninth AC is quite distinct from all of the other known AC subfamilies, and it is not affected by G$\beta y$ proteins or Ca$^{2+}$ (5). Thus, in vivo, when a cell type or tissue expresses various isoforms of AC one may expect that different physiological situations trigger different responses to the same external stimuli depending not only on the type of the receptor and G protein involved but also on the type of adenylyl cyclase to which they are coupled.

As an initial approach to this issue in human and rat myometrium, we first characterized the type(s) of AC involved in myometrial signaling in both species during pregnancy. Then, because AC types II and IV are present in human and rat myometria, we examined the possibility that the positive input to the myometrial AC system emanating from the activated $\alpha_2$-adrenergic receptor (AR) coupled to G$\alpha_2$G$\alpha_3$ proteins as described previously (16) involves G$\beta y$. This was performed by using transducin-$\alpha$ (17) or a synthetic peptide corresponding to the binding site of $\beta y$ in the AC II (18, 19) which tie up the G$\beta y$ released by $\alpha_2$-AR activation. Finally, because AC types V and VI are also strongly expressed in pregnant myometria, we investigated whether submicromolar concentrations of Ca$^{2+}$-attenuate forskolin-stimulated AC activity. Elevation of intracellular Ca$^{2+}$ is precisely the situation that occurs in response to external stimuli in the pregnant myometrium at the time of delivery. Data reported here provide an explanation for the
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EXPERIMENTAL PROCEDURES

Materials—The [3H]cAMP assay system and adenosine-3′,5′-monophosphate (3′,5′-cAMP, 3,000 Ci/mmol) were purchased from Amersham Corp. [3H] Forskolin (31 Ci/mmol) was from NEN Life Science Products. Isoproterenol, clonidine hydrochloride, and all other reagents of the highest grade available were obtained from Sigma. QEHA peptide with 95% purity was synthesized by Eurogentec (Belgium). Transducin-α was kindly donated by Dr. D. T. Wieland, SKEE peptide and the cDNAs encoding for AC types V and VI were kindly provided by Drs. R. Iyengar and J. P. Pieroni. Other types of AC cDNAs were kindly provided as follows: type I, Dr. W. J. Tang; types II and III, Dr. R. R. Reed; type IV, Dr. A. Gilman; type VII, Dr. S. M. Lanier; type VIII, Dr. J. Krupinsky; type IX, Dr. R. Premont.

Animals—Sprague-Dawley rats (250–300 g) were obtained from Iffla Credo (L’Arbresle, France). The females were caged with males overnight, and successful mating was determined by the presence of spermatozoa in the vaginal smear (day 1 of pregnancy). In our breeding colony, parturition occurs between 12 and 19 h on day 22 for 80% of rats (20). Pregnant rats were sacrificed by cervical dislocation at mid-pregnancy (days 14–15) or term (day 22, 18 h). The uterine horns were quickly isolated, cut open lengthwise, and the fetoplacental units re-arranged for transport to the laboratory. Tissue samples were frozen and stored in liquid nitrogen until required.

Human Tissue Collection—Samples of myometrium from preterm (42 weeks gestation) nonlaboring women were taken from the upper border of the uterine incision during elective cesarian sections indicated for cephalo-pelvic disproportion. Tissue samples were frozen and stored in liquid nitrogen until required. This investigation had the approval of the Ethics Committee of INSERM.

Membrane Preparation—Myometrial plasma membranes were prepared from freshly isolated tissues. Briefly, myometrial tissues were homogenized in 10 volumes of ice-cold 10 mM Tris, 250 mM sucrose (pH 7.4). The homogenates were filtered through a double layer of gauze and centrifuged at 20,000 × g for 10 min at 4 °C. Pellets were then resuspended in 50 mM Tris-HCl, 10 mM MgCl₂ buffer (pH 7.4). Protein concentration was determined by the method of Lowry et al. (21). Total protein (20 μg) was used to promote the formation of Gsα.
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RESULTS

Adenylyl Cyclase Expression in Pregnant Myometrium—Types of AC expressed in rat and human myometria were determined by RNA blot analysis using specific cDNA probes for enzyme types I–IX. Brain, which expresses each enzyme type, was used as a positive control for hybridization experiments. Rat lung (AC types II–VI and type IX) or kidney (AC types III, IV, VI, and IX), NIH-3T3 fibroblasts (AC type VI), and DDT-MF2 smooth muscle cells (AC types VI–IX) (for review, see Refs. 3 and 11) were also used as negative or positive controls. Hybridization of poly (A)* RNA (10 µg) isolated from brain (B), human myometrium (Hm), rat myometrium (Rm), lung (L), kidney (K), NIH-3T3 fibroblast (NIH), and DDT-MF2 muscle cells (DDT) were hybridized to a 32P-labeled AC probe (types I–IX), as described under “Experimental Procedures.” cDNA probes were as follows: type I (bovine), nucleotides 736–3191; type II (rat), full-length (4 kb); type III (rat), full-length (4.6 kb); type IV (rat), full-length (3.2 kb); type V (rat), nucleotides 745–1204; type VI (rat), full-length (3.7 kb); type VII (DDT-MF2), nucleotides 3522–3762; type VIII (rat), full-length (4 kb); type IX (mouse), nucleotides 1–957. The position of coelectrophoresed RNA size markers (in kb) is indicated.

Fig. 1. Identification by RNA blot analysis of adenylyl cyclases in pregnant human and rat myometrium. Poly(A)* RNA (10 µg) was hybridized to a 32P-labeled AC probe (types I–IX), as described under “Experimental Procedures.” cDNA probes were as follows: type I (bovine), nucleotides 736–3191; type II (rat), full-length (4 kb); type III (rat), full-length (4.6 kb); type IV (rat), full-length (3.2 kb); type V (rat), nucleotides 745–1204; type VI (rat), full-length (3.7 kb); type VII (DDT-MF2), nucleotides 3522–3762; type VIII (rat), full-length (4 kb); type IX (mouse), nucleotides 1–957. The position of coelectrophoresed RNA size markers (in kb) is indicated.

myometrial membranes in the presence of MgCl2 was saturable at 100 nM [3H]forskolin and reversible at high concentrations of unlabeled forskolin. Specific binding represents 50% of total binding. The time required for half-maximal binding was 10 min, and binding equilibrium at 0 °C was reached at 60 min (data not shown). In the absence of Gpp(NH)p, specific [3H]forskolin binding to myometrial membranes was similar at mid-pregnancy and term with no change of Kd values (Table I). Gpp(NH)p substantially increased both [3H]forskolin binding sites number (× 2-fold) and Kd value at mid-pregnancy, whereas no change was observed at term. These results strongly suggest differences in the number and/or the type of Gs/catalytic subunits complexes which could be formed at both stages of pregnancy.

Influence of GTP, Cholera Toxin, Forskolin, and Isoproterenol on Adenylyl Cyclase Activity in Pregnant Rat Myometrium—As shown in Table II, basal AC activities in mid-pregnant and term myometria were similar. Although GTP increased myometrial AC activity at both stages in a dose-dependent manner, there was significantly more stimulation in myometria taken at mid-pregnancy than from myometria at term (see Vmax in Table II and Fig. 2). Sensitivity of AC, evaluated by half-maximal stimulation, was not different between these two stages (mean value for EC50 = 69.0 ± 1.5 µM). Stimulation with cholera toxin or forskolin also elevated myometrial AC activity but again to a lesser degree at term (Table II). Similar results were found with Gpp(NH)p (data not shown). In contrast, when the activity of the catalytic subunit of AC was evaluated in the presence of Mn2+ (10 mM) and GDPβS (300 µM) no decrease was observed at the last stages of pregnancy. Altogether, these results indicate that the reduced AC stimulability at term is probably caused, at least in part, by a lower level of functional Gs rather than by changes in AC expression. Stimulation of AC was also investigated after activation of the β2-AR/Gs signaling cascade. Maximal response to the agonist isoproterenol was observed at 0.1 mM and was substantially higher in myometria taken at mid-pregnancy (Table II).

Effects of Transducin-α, QEHA Peptide, and SKEE Peptide on Clonidine Potentiation of Isoproterenol-stimulated Adenylyl Cyclase Activity of Myometrial Membranes at Mid-pregnancy—We reported previously (16) that activation of α2-AR/Gs signaling cascade by a micromolar concentration of clonidine resulted in a potentiation of β2-AR/Gs signaling cascade. Maximal response to the agonist isoproterenol was observed at 0.1 mM and was substantially higher in myometria taken at mid-pregnancy (Table II).

TABLE I

| [3H]Forskolin bound | Kd values |
|---------------------|----------|
| fmol/mg protein | nM |
| Gpp(NH)p |
| Day 14 | 31 ± 5 (7) | 68 ± 11 (7) |
| Term | 41 ± 8 (5) | 71 ± 10 (5) |
| +Gpp(NH)p |
| Day 14 | 62 ± 7* (4) | 177 ± 45* (4) |
| Term | 34 ± 5 (4) | 62 ± 10 (4) |

* Statistically significant difference from −Gpp(NH)p (p < 0.05; non-paired Student’s t test).

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concentrations of the QEHA peptide (Table III). Altogether, this suggests that the QEHA peptide, encoding residues 956–982 in the C2a region of AC II, is involved in the modulation of AC activity by Giα and, thus, to prevent potentiation of AC mediated by Giα.

As illustrated in Fig. 3, activation of α2-AR by 0.1 mM clonidine significantly potentiates the isoproterenol-stimulated AC activity by 1.6-fold. In the presence of increasing concentrations of transducin-α, a scavenger of Gbg, effects of transducin-α on clonidine potentiation of isoproterenol-stimulated adenylyl cyclase activity of myometrial membranes at mid-pregnancy. AC activity was assayed by radioimmunoassay as described under “Experimental Procedures” in the presence of isoproterenol 0.1 mM and GTP 0.1 mM plus clonidine 0.1 mM. Transducin-α was used at a final concentration of 50, 100, or 200 nM. Basal adenylyl cyclase activity was 77 ± 7 pmol of cAMP/mg of protein/10 min. Results are expressed as the mean ± S.E. of the number of determinations indicated in parentheses. α indicates a statistically significant difference from isoproterenol; b indicates a statistically significant difference from isoproterenol plus clonidine (p < 0.05; nonpaired Student’s t test).

These results confirm the Gβγ involvement in α2-AR-mediated potentiation of AC.

Effects of Micromolar Concentrations of Calcium on Adenylyl Cyclase Activity in Pregnant Myometrial Membranes—Among the seven isoforms of AC identified in pregnant myometrium, types V and VI are characterized by an activity regulated by submicromolar concentrations of Ca2+ (2, 31, 32). Thus, to

| Stimulators | Day 14 of pregnancy | Term |
|-------------|---------------------|------|
| None (basal activity) | 71 ± 4 (8) | 69 ± 3 (3) |
| GTP | 137 ± 3 (19) | 103 ± 2*(19) |
| Forskolin + GTP | 254 ± 7 (8) | 192 ± 9*(6) |
| Cholera toxin + GTP | 280 ± 10 (10) | 213 ± 9*(10) |
| Isoproterenol + GTP | 175 ± 15 (10) | 136 ± 6*(8) |

*Statistically significant difference between the two stages of pregnancy (p < 0.05; nonpaired Student’s t test).

**Fig. 2.** Concentration-response curves of GTP on adenylyl cyclase activities in rat myometrial plasma membranes. AC activity was assayed by radioimmunoassay as described under “Experimental Procedures;” and the results are expressed as total activity (pmol of cAMP/mg of protein/10 min). Basal adenylyl cyclase activity on day 14 of pregnancy or term was 71 ± 4 and 69 ± 3 pmol of cAMP/mg of protein/10 min, respectively. Each data point is the mean of four experiments.

**Fig. 3.** Effects of transducin-α on clonidine potentiation of isoproterenol-stimulated adenylyl cyclase activity of myometrial membranes at mid-pregnancy. AC activity was assayed by radioimmunoassay as described under “Experimental Procedures” in the presence of isoproterenol 0.1 mM and GTP 0.1 mM plus clonidine 0.1 mM. Transducin-α was used at a final concentration of 50, 100, or 200 nM. Basal adenylyl cyclase activity was 77 ± 7 pmol of cAMP/mg of protein/10 min. Results are expressed as the mean ± S.E. of the number of determinations indicated in parentheses. a indicates a statistically significant difference from isoproterenol; b indicates a statistically significant difference from isoproterenol plus clonidine (p < 0.05; nonpaired Student’s t test).

**Fig. 4.** Effects of QEHA and SKEE peptides on clonidine potentiation of isoproterenol-stimulated adenylyl cyclase activity of myometrial membranes at mid-pregnancy. AC activity was assayed by radioimmunoassay as described under “Experimental Procedures” in the presence of 0.1 mM isoproterenol and 0.1 mM GTP plus 0.1 mM clonidine. QEHA and SKEE peptides were used at a final concentration of 25, 50, or 100 nM. Basal adenylyl cyclase activity was 81 ± 7 pmol of cAMP/mg of protein/10 min. Results are expressed as the mean ± S.E. of the number of determinations indicated in parentheses. α indicates a statistically significant difference from isoproterenol; b indicates a statistically significant difference from isoproterenol plus clonidine (p < 0.05; nonpaired Student’s t test).
Effects of increasing concentrations of QEHA peptide on basal and isoproterenol-stimulated adenylyl cyclase activities of myometrial membranes at mid-pregnancy

AC activity, in the presence or absence of QEHA peptide, was measured by radioimmunoassay as described under “Experimental Procedures.” Values are expressed as total activity (pmol/mg of protein/10 min). Results are the means ± S.E. of three independent determinations.

| Adenylyl cyclase activity | QEHA peptide |
|---------------------------|--------------|
| Control                   | 50 nM        |
|                           | 100 nM       |
|                           | 200 nM       |
| Basal                     | 93 ± 4       |
|                           | 99 ± 3       |
|                           | 110 ± 6      |
|                           | 109 ± 5      |
| Isoproterenol (0.1 mM)    | 121 ± 3      |
|                           | 130 ± 2      |
|                           | 119 ± 6      |
|                           | 123 ± 2      |

FIG. 5. Inhibition curve of myometrial adenylyl cyclase activity by increasing free Ca\(^{2+}\) concentrations at mid- and term pregnancy. Endogenous Ca\(^{2+}\) and calmodulin were depleted with EGTA 0.2 mM. AC activity was stimulated by 0.1 mM forskolin. Enzyme activity was assayed as described by Salomon et al. (25; see “Experimental Procedures”). Mean basal enzyme activity was 115 ± 10 pmol/mg of protein/10 min; AC activity in presence of 0.1 mM GTP was 187 ± 18 pmol/mg of protein/10 min on day 14 versus 140 ± 53 pmol/mg of protein/10 min at term; AC activity in the presence of 0.1 mM forskolin was 828 ± 110 pmol/mg of protein/10 min on day 14 versus 685 ± 123 pmol/mg of protein/10 min at term. The inset indicates parameters of calcium inhibition of forskolin-stimulated AC activity in rat pregnant myometrial membranes. Data represent the mean ± S.E. of six separate experiments.

FIG. 6. Localization of adenylyl cyclase (type II) in myometrial smooth muscle and in adult rat brain by in situ hybridization. Sections were hybridized to a 35S-labeled antisense oligonucleotide probe as described under “Experimental Procedures.” Bright-field photomicrographs of pregnant rat myometrium on mid-pregnancy were hybridized with the radiolabeled probe in the absence (panel A) or in the presence (panel B) of a 100-fold molar excess of the unlabeled probe, treated with Ilford K-5 emulsion and counterstained with cresyl violet acetate. CM, circular muscle; LM, longitudinal muscle (∗× 450). Panel C, dark-field photomicrograph of rat brain sections showing the distribution of type II AC in the hippocampus. DG, dentate gyrus; PC, pyramidal cells (∗× 24).

DISCUSSION

This study reports for the first time that pregnant human and rat myometria express transcripts encoding distinct populations of AC. Indeed, using Northern blot analysis, we detected AC II–VI and IX in both human and rat myometria, whereas type VII was present only in rat. The absence of detectable levels of types I and VIII mRNA is consistent with previous findings showing that these two enzymes are brain-specific. Although it is generally considered that all the structures that expressed more than one AC type contain several cell types, it is important to note that in the myometrium, smooth muscle cells present a great abundance and are distributed evenly throughout the longitudinal and circular layers. Only few other cell types (vascular or conjunctive) are observed. AC mRNA detected in this study using in situ hybridization derived mainly from myometrial cells as exemplified in Fig. 6, A and B, illustrating the specific expression of AC type II in smooth muscle cells. Furthermore, in agreement with previous results (29, 33), control slides of rat brain hybridized in the same conditions indicated a strong expression of AC II in pyramidal cells and dentate gyrus of hippocampus (Fig. 6C). All of these observations suggested to us that Giβγ-activated (types II and IV and presumably VII) and Ca\(^{2+}\)-inhibitable (types III, V, and VI) AC expressed in human and rat myometrium might contribute to physiological processes sensitive to G proteins and Ca\(^{2+}\)-dependent regulation of the cAMP generation in this tissue.

To estimate the total levels of AC in myometria from mid-pregnant and delivering rats, we measured the maximal levels
of high affinity [3H]forskolin binding sites ($B_{max}$). In the presence of Gpp(NH)p, our results demonstrated an increase of the [3H]forskolin binding sites at mid-pregnancy but not at term. Because it was proposed that the guanine nucleotide-stimulated high affinity binding of [3H]forskolin represents binding to the G$_s$-activated form of AC, one may suggest that the G$_s$/catalytic unit complexes are reduced at term. Potential differences in the affinity of this ligand ($K_d$) observed in mid-pregnant rat myometrium could mean either differences in the interaction of [3H]forskolin with some isoforms of AC present in the myometrium or differential expression of isofoms according to the stage of pregnancy. Although our results show that the expression of AC transcripts in rat myometrium over the

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...the expression of AC transcripts in rat myometrium over the second half of pregnancy remains stable, we cannot exclude possible changes at the protein levels. At the present time, this issue cannot be settled because no specific antibody that allows to quantify directly each AC type is available. Interestingly, the decrease of [3H]forskolin binding sites number reported at term in the presence of Gpp(NH)p correlates well with the decline of forskolin-stimulated AC activity. Furthermore, the reduced ability of guanine nucleotides or chola toxin (acting at the G level) to stimulate myometrial AC activity at term and the fact that Mn$^{2+}$/GDP/$\beta\gamma$ (acting solely at the catalytic unit of the AC, 34) has no different regulatory effects, points to a defect in the availability or functionality of regulatory G$_s$ proteins rather than a reduction in the catalytic subunits of AC. This conclusion supports previous data of our laboratory establishing that amounts of chola toxin-catalyzed ADP-ribosylated G$_s$,G$_\beta\gamma$ subunits diminished by 3-fold at delivery versus pregnancy (35). In addition, as shown by radioligand binding experiments, the reduced response to isoproterenol at term is not caused by changes in total $\beta$-AR number (28) but is the consequence of G$_s$ protein uncoupling (24).

Previously, we have shown that catecholamines acting through $\alpha_2$-AR/G$_{12,3}$ proteins signaling pathway potentiate $\beta$-AR-stimulated AC activity in rat myometrium at mid-pregnancy (16). These data suggested a tight interplay between the two adrenergic pathways in the regulation of AC types II and IV through $\beta\gamma$ complexes according to the findings of Tang and Gilman (6), Gao and Gilman (7), and Federman et al. (36). Here, the demonstration that the positive input emanating from the activated $\alpha_2$-AR involves $\beta\gamma$ complexes of G proteins has been observed in rat myometrium (37) was achieved using transducin-$\alpha$ and QEHA peptide: both factors abolished the potentiation of AC activity induced by the $\alpha_2$-AR agonist clenodidine. In the same work (16), we reported a switch in the stimulatory versus inhibitory input to AC population from the $\alpha_2$-AR/G$_{12,3}$ protein-signaling pathway at late term. The idea that changes in AC isofrom expression might underlie such a versatility of the $\alpha_2$-AR signal is highly speculative inasmuch as it is not possible to demonstrate that some AC types (specially types II and IV) are the most prevalent isofoms in the mid-pregnant myometrium compared with late term myometrium. At this latter period, no substantial modification in the amounts of specific types of AC transcripts as well as no alteration in the basal activity of the AC system have been observed. So, in the parturient rat myometrium, when the balance among G$_s$, G$_\alpha$, and G$_s$ proteins was changed (35, 37) we suggest the possibility that distinct patterns of responsiveness of the myometrial AC population may account for the switch between the two types of input (positive and negative) initiated by $\alpha_2$-AR activation. Interestingly, a decline of immunodetecter $\beta$ subunits of G proteins which is expected to reflect the status of the $\beta\gamma$ complex (38) paralleled the 1.7-fold decrease of G$_2$,G$_1$ subunit in the late pregnant rat myometrium (37, 38). It is then conceivable to propose that the reduced availability of Gi$\beta\gamma$ complexes together with the reduced levels of functional G$_s$,G$_\alpha$ protein may underlie the loss of AC potentiation observed at term in response to $\beta_2$/G$_s$-AR activation. On the other hand, the role of G$_s$ protein-coupled $\alpha_2$-AR in mediating AC inhibition in the late pregnant myometrium has been well documented in rats (16) and human (39). To date, the inhibitory regulation of AC type II through G$_s$ proteins still remains controversial since the data reported by Chen and Iyengar in COS7 cells transfectants (40) and the recent work of Lanier's laboratory in collaboration with us on DDT$_1$,MF2 cells AC II transfectants (11) are not in agreement with other findings in SK9 cell membranes (4). Nevertheless, AC types III, V, and VI expressed in rat and human myometrium are highly sensitive to inhibition by G$_i$, G proteins (4, 40) and could then present possible targets to $\alpha_2$-AR/G$_i$, protein-mediated inhibition at term. The presence of AC types V and VI is also consistent with the observation that physiological relevant concentrations of Ca$^{2+}$ inhibit basal and stimulated AC population in pregnant myometrial membranes of rat (our present work) and human (41). This is in line with preliminary evidences showing that these enzymes are regulated directly by submicromolar concentrations of Ca$^{2+}$ (2, 31, 32). As demonstrated for cardiac adenylyl cyclases (42), the inability of maximally stimulating concentrations of Mg$^{2+}$ to overcome Ca$^{2+}$ inhibition (data not shown) indicates that Ca$^{2+}$ concentrations in the $\mu$m range are not competitive with Mg$^{2+}$ (i.e. high affinity effect) by contrast with millimolar concentrations of Ca$^{2+}$ (43). Half-maximal inhibition value reported in this work is also in agreement with values found for cardiac muscle (42, 44) or the aorta smooth muscle (45) AC system. Interestingly, these Ca$^{2+}$ concentrations at which AC inhibition is reported are in the range of those required to activate myosin light chain phosphorylation (46) and correspond also to Ca$^{2+}$ concentrations measured in myometrial membranes from rats close to term treated with the uterotonic agent oxytocin (47). In addition, Ca$^{2+}$ can affect also indirectly AC activity because inhibition of AC type III activity through its phosphorylation by calmodulin-casein kinase II is mediated by intracellular increase of Ca$^{2+}$ (15). In conditions prevailing in vivo in the pregnant myometrium at term when intramyometrial submicromolar concentrations of Ca$^{2+}$ rise, such regulation may also provide a mechanism for attenuation of hormone-stimulated cAMP generation. Alternatively, Lanier's group together with our laboratory (11) have established that the stimulatory/inhibitory input to the AC types II and IV initiated through $\alpha_2$-AR activation is modified differentially by protein kinase C-mediated effects. In particular, activation of protein kinase C in AC IV-transfected DDT$_1$,MF2 cells eliminates the stimulatory input due to $\alpha_2$-AR activation. All of these observations indicate that the rise of intracellular Ca$^{2+}$ occurring at term in response to external stimuli (oxytocin, prostaglandin F$_2\alpha$, endothelins, acetylcholine through M$_3$-muscarinic receptors, norepinephrine through $\alpha_1$-AR or $\alpha_2$-AR) would reduce the synthesis of the smooth muscle relaxation mediator, i.e. cAMP, thereby allowing myometrial cells to contract.

In conclusion, analysis of the functional properties of the AC population identified in the myometrium demonstrates for the first time that $\beta\gamma$ complexes and Ca$^{2+}$ are two main regulators of the AC/cAMP cascade during pregnancy in the rat. Because of similarities between human and rat myometrium with regard to AC types, as well as G proteins and membrane receptors, we postulate similar physiological regulations of the AC/cAMP system in human. Thus, cross-talks between various hormonal signals that are routed differentially through G$_s$, G$_i$, or G$_s$ proteins according to the stage of pregnancy may dictate the final response of the AC/cAMP system and consequently the contractile state of the uterus.
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