In vivo stimulation of the β_2-adrenergic pathway increases expression of the G_i proteins and the α_{2A}-adrenergic receptor genes in the pregnant rat myometrium

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Abstract

Cross-regulations between G_s and G_i mediated pathways controlling the adenylyl cyclase activity have been clearly demonstrated *in vitro*. To elucidate whether activation of the β-adrenergic pathway in the pregnant myometrium might affect G_i proteins and α_2-adrenergic receptors (ARs), we treated late pregnant rats from day 18 to day 21 with twice-daily administration of isoproterenol (8 mg/kg). This treatment increased myometrial cAMP levels and led after 76 h to a significant and maximal rise in the immunoreactive amount of myometrial $G_{i\alpha2}$ and $G_{i\alpha3}$ proteins (1·4- and 1·7-fold respectively) associated with a parallel increase of the steady-state levels of both $G_{i\alpha2}$ and $G_{i\alpha3}$ mRNA (1·6- and 1·9-fold respectively). Propranolol antagonized this response indicating the implication of the β-adrenergic pathway. Nuclear run-on assays demonstrated that isoproterenol enhanced respectively by 1·3- and 1·2-fold the transcription rate of the $G_{i\alpha2}$ and $G_{i\alpha3}$ genes. Quantification of myometrial α_2-ARs by [^{3}H]rauwolscine binding revealed that the total number of receptors was also increased at 76 h by 1·7-fold when compared with controls, with no change in the affinity of the α_2-ARs for the ligand. This effect was antagonized by propranolol. Quantification of both α_{2A}- and α_{2B}-subtypes by Northern blotting analysis demonstrated that this elevation was due to a selective increase of the α_{2A}-subtype mRNAs. The present results indicate that *in vivo* stimulation of the β-adrenergic pathway by isoproterenol increases both $G_{i\alpha2}/G_{i\alpha3}$ and α_{2A}-AR expression in the pregnant rat myometrium. The possible contribution of such a mechanism in pregnancy-related changes of both entities is discussed.

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Introduction

It is well established that the adrenergic system is a powerful mechanism which influences the contractile state of the myometrium during pregnancy in response to hormonal status (Marshall 1981). In a large variety of animals, noradrenaline produces inhibitory and excitatory effects on contractility through the activation of myometrial β- and α_1-adrenergic receptors (ARs) respectively.

The β-ARs couple via G_s to myometrial adenylyl cyclase to increase intracellular levels of cAMP and to activate cAMP-dependent protein kinases which lead to myometrial relaxation through effects on intracellular Ca^{2+} concentration and myosin light chain kinase. Both β_1- and β_2-ARs coexist in the myometrium with a higher proportion of the β_2-subtype (approximately 85% of the total β-AR population). During pregnancy, this subtype is selectively up-regulated by progesterone at the level of gene transcription (Vivat *et al.* 1992). In addition, progesterone has been shown to stabilize the high affinity state of the receptor, thus increasing the coupling of the β_2-ARs to adenylyl cyclase (Cohen-Tannoudji *et al.* 1991) and facilitating uterine quiescence. In contrast, the α_1-ARs play an important role in the mechanism of parturition, as supported by several lines of evidence (Legrand & Maltier 1986, Pennefather *et al.* 1993). At delivery, myometrial contractions are initiated primarily by a rise of intracellular Ca^{2+}. Recent work of our laboratory indicates that the α_{1B}-subtype is one of the determinants which activates the $G_{q\alpha}$-$G_{11\alpha}$/phospholipase C signaling pathway in the preparturient rat myometrium.

The role of the α_2-ARs seems to be complex in the control of pregnancy-related changes of the adenylyl cyclase system and uterine contractility. Indeed, according to the stage of pregnancy, clonidine, a potent α_2-adrenergic agonist, either potentiates or inhibits the β_2-AR-stimulated myometrial adenylyl cyclase pathway by coupling to both G_{i2} and G_{i3} (Mhaouty *et al.* 1995), the only two forms of inhibitory proteins expressed in rat

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myometrium (Cohen-Tannoudji *et al.* 1995). Potentiation of cAMP production by the α_2/G_i pathway at mid-pregnancy is related to the β-AR activation of G_s, high levels of G_{i3} and G_{i2} proteins and the presence of type II and IV adenylyl cyclases in the rat myometrium (Mhaouty *et al.* 1995). At the end of pregnancy, the stimulabilities of the cAMP generating system by isoproterenol (Litime *et al.* 1989, Cohen-Tannoudji *et al.* 1991) and α_2 agonists (Mhaouty *et al.* 1995) decline. Concomitantly, a reduction in the myometrial levels of ADP-ribosylated G_s and G_{i3} as well as in the α_{2A}-AR subtype expression is observed (Elwardy-Mérézak *et al.* 1994, Cohen-Tannoudji *et al.* 1995, Mhaouty *et al.* 1995).

Cross-regulations between G_s and G_i protein-mediated pathways have been clearly demonstrated in several *in vitro* studies. The observations that activation of the stimulatory pathway of adenylyl cyclase by the β-adrenergic agonist isoproterenol and forskolin or by cAMP analogs respectively increases G_i proteins in S49 cells (Hadcock *et al.* 1990) and α_2-ARs in HT29 cells (Sakaue & Hoffman 1991) has led to the proposal of a role of cAMP in these cross-regulatory mechanisms. We wondered whether such regulations might affect one or several entities of the myometrial adenylyl cyclase pathway during pregnancy. In the present work, we thus explored, in the intact pregnant rat, the effects of a sustained activation of the β_2-AR pathway on the regulation of the expression of myometrial G_i proteins and α_2-AR genes.

Materials and Methods

Chemicals and reagents

cAMP [^{3}H] assay system, [^{3}H]rauwolscine (88 Ci/mmol) and [α-^{32}P]UTP (400 Ci/mmol) were purchased from Amersham Corp. (Les Ulis, France); [α-^{32}P]dCTP (3000 Ci/mmol), G-protein antisera and $G_{i\alpha2}$ synthetic oligodeoxynucleotide probe were obtained from Dupont–New England Nuclear (Le Blanc Mesnil, France); (±)-isoproterenol-HCl and (±)-propranolol-HCl were from Sigma (L'Isle d'Abeau, France); cesium-trifluoroacetate and prepacked oligo(dT)-cellulose columns were from Pharmacia LKB Biotechnology Inc. (Saclay, France). All other compounds were of molecular biology grade or of the highest grade commercially available.

Animals and treatments

Sprague–Dawley rats (250–300g) were obtained from Iffa-Credo (L'Arbresle, France). They were maintained in accordance with the guidelines for care and use of laboratory animals (NIH Guide). 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 1200 and 1900 h on day 22 for 80% of rats.

The rats were divided into three experimental groups which were treated from day 18 to day 21 of pregnancy with either 0·9% NaCl as control, or (±)-isoproterenol-HCl 8 mg/kg, or (±)-isoproterenol-HCl in combination with (±)-propranolol-HCl 40 mg/kg. Rats received twice-daily injection (i.p.) of saline or drugs. Propranolol was administered 30 min before isoproterenol according to the method of Kimura *et al.* (1993). Animals were killed by cervical dislocation at the indicated times after the first injection. Myometrial tissues were then immediately removed, trimmed of fat and connective tissues and scraped from adherent endometrium.

Radioligand receptor binding assay

Myometrial plasma membranes were prepared as described previously (Legrand *et al.* 1987). Protein concentration was determined by the method of Schacterle & Pollack (1973). Total binding was determined by incubating 200 μg of membrane suspension with 1–30 nM [^{3}H]rauwolscine in 150 μl of 50 mM Tris–HCl (pH 7·4) containing 10 mM $MgCl_2$, 50 μM ascorbic acid and 5 μM pargyline. Non-specific binding was determined in the presence of 10 μM phentolamine. After 20 min of incubation at 25 °C, 5 ml of ice-cold buffer was added to each tube and immediately filtered through a Whatman GF/C glass fiber filter. The retained radioactivity was determined by liquid scintillation in a Spectrometer 1214 Rackbeta (Pharmacia LKB Biotechnology Inc.) at 50% efficiency.

Immunoblotting of G-protein subunits

Preparation of myometrial membrane fractions, SDS-PAGE and immunoblotting with AS/7 (anti-$G_{i\alpha2}/G_{i\alpha1}$) or EC/2 (anti-$G_{i\alpha3}/G_{o\alpha}$) antisera were performed according to the methods previously described (Elwardy-Mérézak *et al.* 1994, Cohen-Tannoudji *et al.* 1995). Antibody complexes were detected using a chemiluminescent method (ECL; Amersham Corp.) and quantified by scanning densitometry on a GS300 densitometer (Hoefer Scientific Instruments, San Francisco, CA, USA). Data were obtained under conditions where a linear relationship existed between the intensity of the ECL signal and the amount of protein loaded (data not shown).

RNA preparation and Northern blotting

Total RNA was extracted from myometrial tissues by the cesium-trifluoroacetate gradient method (Okayama *et al.* 1987) and poly(A)$^+$ RNA was purified using oligo(dT) columns. Fifteen micrograms total RNA (G blotting) or 10 μg poly(A)$^+$ RNA (α_2-AR blotting) were denatured by formaldehyde, fractionated by 1% agarose gel electrophoresis and transferred to GeneScreen-Plus membranes (Dupont–New England Nuclear) by overnight capillary

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blotting. The blots were prehybridized in 45% formamide, 4 × SSC, 5 × Denhardt's solution, 0·5% SDS, 75 µg/ml denatured salmon sperm DNA, at 42 °C for 4 h. Hybridization was performed overnight at 42 °C in the same buffer containing 10% dextran sulfate and 10^6 c.p.m./ml ^{32}P-radiolabeled probes. The synthetic oligodeoxynucleotide $G_{i\alpha 2}$ probe was complementary to bases encoding amino acids 172–184 of $G_{i\alpha 2}$ and the $G_{i\alpha 3}$ probe corresponded to a 625 bp *Eco*RI-*Eco*RV fragment of the 3′ non-coding end of $G_{i\alpha 3}$, as described elsewhere (Cohen-Tannoudji *et al.* 1995). Probes specific for each α_2-AR subtype were chosen from regions coding for the putative third intracellular loop of the receptors since these sequences are divergent between the different subtypes. The 333 bp *KpnI-XmnI* fragment was derived from the rat brain α_{2A} cDNA and a 279 bp sequence of the α_{2B}-subtype (nucleic acids 981 to 1259) was amplified from rat genomic DNA using PCR as described previously (Mhaouty *et al.* 1995). A rat cyclophilin ^{32}P-radiolabeled probe was used as internal control for RNA hybridization.

Measurement of run-on gene transcription on isolated nuclei

Myometria from day 21 control rats and from rats treated by injections of isoproterenol for 76 h were used for nuclei isolation. Nuclei were prepared according to the method of McKnight & Palmiter (1979) as described previously (Vivat *et al.* 1992). The isolated nuclei were resuspended in 50 mM Tris–HCl (pH 8·3), 40% glycerol, 5 mM $MgCl_2$ and 0·1 mM EDTA and stored at −80 °C. Nuclei (2×10^7) were incubated for 30 min at 30 °C with 250 µCi [α-^{32}P]UTP (400 Ci/mmol) in a 200 µl mixture prepared as described by Antras *et al.* (1991). The mixture was then treated with DNase I and proteinase K (10 mg/ml) as previously described by Vandenbrouck *et al.* (1994); nascent RNAs were extracted with 8 M guanidine–HCl (pH 5·0) containing 20 mM $Na(CH_3CO_2)$, 1 mM dithiothreitol and 0·5% (w/v) lauryl sarcosine. The radiolabeled RNAs (50×10^6 c.p.m.) were hybridized at 42 °C for 72 h with rat $G_{i\alpha 2}$, $G_{i\alpha 3}$ or cyclophilin cDNAs (4 µg) immobilized to a nitrocellulose membrane. A specific 1750 bp *Eco*RI fragment was used for $G_{i\alpha 2}$. cDNAs for $G_{i\alpha 3}$ and cyclophilin were equivalent to the probes used in Northern blotting analysis. The membranes were washed twice with 300 mM NaCl, 20 mM NaH_2PO_4 and 2 mM EDTA at 37 °C for 15 min and then treated with 5 mg/ml of RNase A at 37 °C for 30 min in the same buffer. Membranes were exposed to Kodak-X-Omat-AR films at −80 °C for 1–5 days. After autoradiography, each slot was cut out and quantified by liquid scintillation spectrometry.

Determination of myometrial cAMP content

Myometrial cAMP content was measured according to the method of Humphreys-Beher & Schneyer (1986). Fresh myometrial tissue was homogenized in the presence of 1 mM 3-isobutyl-1-methylxanthine and then centrifuged for 30 min at 30 000 ***g***. Soluble material of the resulting supernatant was extracted by the addition of an equal volume of 12% trichloroacetic acid (TCA) and insoluble proteins were removed by centrifugation at 10 000 ***g*** for 15 min. Residual TCA was removed from the supernatant prior to assay by three extractions with diethyl ether. cAMP content was determined by RIA (Cyclic AMP [^{3}H] assay system, Amersham).

Table 1 Effect of isoproterenol on levels of cAMP in pregnant rat myometrium. Myometrial tissues were excised at the indicated times after isoproterenol injection and cAMP content was immediately determined as described in Materials and Methods. Values are mean ± S.E. from three to five separate experiments. [a] Indicates a statistically significant difference from controls D18 ($P<0·05$; non-paired Student's *t*-test)

Time (min)	**cAMP** (pmol/mg protein/10 min)
0 (basal)	32 ± 6
15	29 ± 2
30	57 ± 8[a]
90	51 ± 8

Results

Effects of isoproterenol injections on cAMP content in the pregnant rat myometrium

Isoproterenol injection resulted in a time-dependent increase of myometrial cAMP content with a maximal effect observed after 30 min (Table 1).

Effects of isoproterenol on G_i protein expression in the pregnant rat myometrium

The expressions of $G_{i\alpha 2}$ (40 kDa) and $G_{i\alpha 3}$ (41 kDa), the predominant isoforms of G_i in the pregnant rat myometrium, were assessed at the protein and mRNA levels by the use of quantitative Western and Northern blotting. In comparison with control rats on day 18 or day 21 of pregnancy, a 76 h treatment with isoproterenol significantly increased the steady-state levels of both $G_{i\alpha 2}$ and $G_{i\alpha 3}$ (100 to 144 ± 10% or 170 ± 4% respectively, Fig. 1). No significant change was observed at an earlier stage of isoproterenol treatment. G_i increase was antagonized by the β-adrenergic antagonist propranolol, demonstrating the implication of the β-AR pathway. Propranolol by itself had no effect on G-protein expression (data not shown).

The ^{32}P-oligodeoxynucleotide probe encoding $G_{i\alpha 2}$ hybridized to a single band of 2·4 kb and the $G_{i\alpha 3}$ radiolabeled cDNA detected a single band of 3·5 kb which

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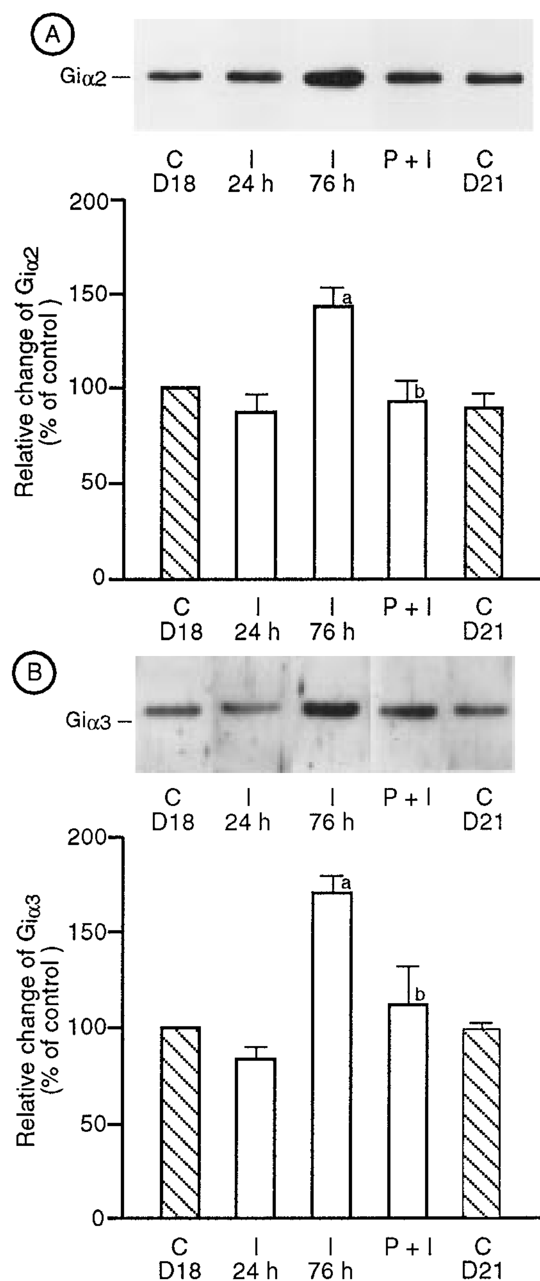
is in agreement with the known size of the transcripts (Cohen-Tannoudji *et al.* 1995). The time-course of changes in $G_{i\alpha2}$ and $G_{i\alpha3}$ mRNA levels following repeated administration of isoproterenol *in vivo* revealed that β_2-adrenergic stimulation led to a slow and gradual increase in the mRNA expression of both myometrial $G_{i\alpha}$ subunits (Fig. 2). Maximal increase (+58 ± 6% for $G_{i\alpha2}$; +85 ± 10% for $G_{i\alpha3}$) was reached at 76 h of treatment. These results agree well with the changes that occurred in steady-state levels of expressed proteins.



Run-on transcription assays (Fig. 3) demonstrated that repeated injections of isoproterenol enhanced by 30 ± 7% and 23 ± 3% respectively the transcription rate of the $G_{i\alpha2}$ and $G_{i\alpha3}$ genes in comparison with control myometria of the same stage of pregnancy. $G_{i\alpha2}$ and $G_{i\alpha3}$ genes are thus transcriptionally activated in a coordinated manner in the pregnant rat myometrium after isoproterenol injections.

Effects of isoproterenol on myometrial α_2-AR levels

The density of α_2-ARs in isoproterenol-treated rats, compared with controls, was quantified using [^{3}H]rauwolscine. The total number of receptors was unchanged during the first 24 h of exposure to isoproterenol and was maximally increased by 165 ± 18% at 76 h of treatment (Fig. 4). Thus at this time, the number of α_2-ARs in treated rats was increased by 103 ± 14% compared with control values at the same stage of pregnancy. This change was abolished in the presence of propranolol which antagonized the effect of isoproterenol on α_2-ARs (72 ± 5 fmol/mg protein in 76 h isoproterenol-treated rats, 38 ± 4 fmol/mg protein in isoproterenol and propranolol treated rats and 35 ± 3 fmol/mg protein in day 21 control rats). Over the treatment period, there was no change in the affinity of the α_2-ARs for [^{3}H]rauwolscine (mean K_d value in isoproterenol-treated rats: 8·1 ± 1·0 nM vs 7·8 ± 1·2 nM in control rats).

Using Northern blotting analysis, we detected in isoproterenol-treated rats a gradual increase of the density of the 3·8 kb mRNA corresponding to the α_{2A}-ARs (Fig. 5), reaching a maximal value at 76 h of treatment. In contrast, in control pregnant rats, we did not observe any change in α_{2A}-mRNA abundance between day 18 and day 21. The 4·2 kb message encoding the α_{2B}-subtype was increased after 76 h of treatment and this increase was consistent with the one measured in control rats between day 18 and day 21 of pregnancy (Fig. 5). Thus, repeated

Figure 1 Immunoblot analysis of $G_{i\alpha2}$ and $G_{i\alpha3}$ in myometrial membranes of control pregnant rats or following administration of isoproterenol. Pregnant rats were twice-daily administered isoproterenol (I), or isoproterenol and propranolol (P+I) from day 18 to day 21 of pregnancy. Controls rats at day 18 and day 21 of pregnancy (C D18, C D21) were treated with vehicle (0·9% NaCl). Myometrial membrane fractions were subjected to SDS-PAGE and the separated proteins transferred to nitrocellulose for immunoblotting. The blots were probed with antibody selective for either $G_{i\alpha2}$ (AS/7, panel A) or $G_{i\alpha3}$ (EC/2, panel B) (final dilution 1:1000). Inserts are representative autoradiograms for 40 kDa immunoreactive $G_{i\alpha2}$ and 41 kDa immunoreactive $G_{i\alpha3}$. Quantitative data are expressed as percent of control D18. The data shown are the mean values ± S.E. of three to ten separate experiments; [a] indicates a statistically significant difference from controls D18 and D21; [b] indicates a statistically significant difference from 76 h isoproterenol (P<0·05, non-paired Student's t-test).

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injections of isoproterenol in pregnant rats led to a selective increase of α_{2A}-AR expression in the myometrium.

Discussion

Our present data establish that sustained activation of the β_2-AR pathway by twice-daily injections of isoproterenol from day 18 to day 21 of pregnancy leads to a concomitant increase of $G_{i\alpha2}/G_{i\alpha3}$ subunits and α_{2A}-AR numbers in the myometrium.

Administration of isoproterenol effectively induced an increase of myometrial cAMP and we present clear evidence that changes in $G_{i\alpha2}/G_{i\alpha3}$ and α_{2A}-ARs may be brought about by activation of the β_2-AR signaling cascade since, in the presence of propranolol, a potent β-adrenergic antagonist, isoproterenol failed to induce these changes.

To understand the molecular mechanisms underlying the increase of G_i protein expression, we evaluated G_i mRNA levels and gene transcription. Analysis of the time-course of $G_{i\alpha3}$ and $G_{i\alpha2}$ mRNA levels in isoproterenol-treated rats demonstrated a progressive increase in their steady-state levels which correlated well with the increase in the immunoreactive amount of $G_{i\alpha3}$ (1·7-fold) and $G_{i\alpha2}$ (1·4-fold). Measurement of the relative rate of transcription by nuclear run-on assays revealed that isoproterenol treatment resulted in an enhancement of the transcription rate of the $G_{i\alpha3}$ (1·2-fold) and $G_{i\alpha2}$ (1·3-fold) genes. Such a result is in accordance with that reported in the rat heart by Eschenhagen *et al.* (1991, 1992) who showed that the increase of $G_{i\alpha2}$ mRNA levels after *in vivo* isoprenaline infusion was due to an activation of gene transcription (Müller *et al.* 1993). So, our present work and that of Eschenhagen's group extend some of the *in vitro* observations made previously on a canine kidney cell line (Rich *et al.* 1984), in cultured rat heart muscle (Reithmann *et al.* 1989) and S49 mouse lymphoma cells (Hadcock *et al.* 1990). However, up-regulation of $G_{i\alpha2}$ levels in response to sustained activation of the stimulatory pathway is not a general feature since it has not been observed in NG 108–15 cells exposed to prostaglandin (PG) E_1 or forskolin (McKenzie & Milligan 1990) or in the human neuroblastoma cell line SK-N-MC treated with isoprenaline (Michel *et al.* 1993). Despite these discrepancies, it had been argued that enhanced transcriptional activity of the human $G_{i\alpha2}$ gene depends on the presence of a cAMP response element like AP2 (Weinstein *et al.* 1988). In addition, in S49 mouse lymphoma cells, Hadcock *et al.* (1990) demonstrated that activation of the stimulatory

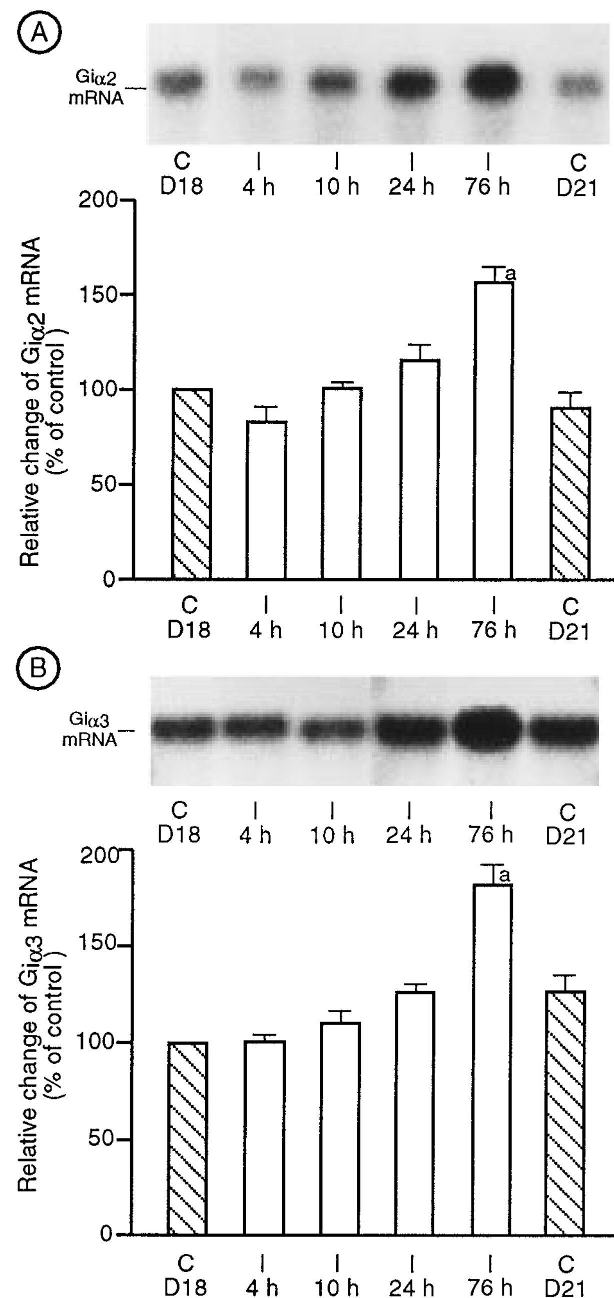

Figure 2 Time course of changes in $G_{i\alpha2}$ and $G_{i\alpha3}$ mRNA values in myometrial membranes of control pregnant rats or following administration of isoproterenol. Pregnant rats were twice-daily injected from day 18 to day 21 of pregnancy with isoproterenol (I). Control rats from day 18 to day 21 of pregnancy (C D18, C D21) were treated with vehicle (0·9% NaCl). mRNA values were determined by Northern blotting using probes for $G_{i\alpha2}$ (panel A) and $G_{i\alpha3}$ (panel B). A rat cyclophilin ^{32}P-radiolabeled probe was used as an internal control. Inserts are representative autoradiograms for $G_{i\alpha2}$ (2·4 kb) and $G_{i\alpha3}$ (3·5 kb) transcripts. The autoradiograms were scanned using a densitometer and quantitative $G_{i\alpha}$ mRNA values are expressed as percent of control D18. The data shown are the mean values ± S.E. of three to six separate experiments; [a] indicates a statistically significant difference from controls D18 and D21 (P<0·05, non-paired Student's t-test).

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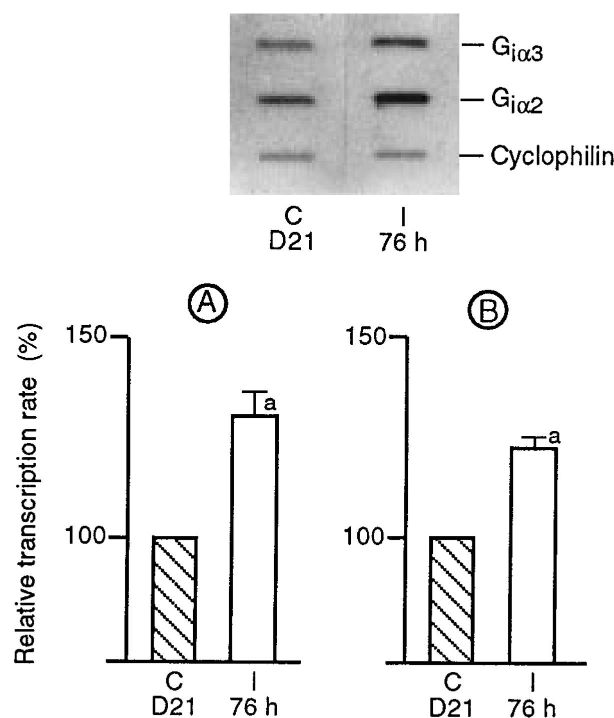

Figure 3 Relative transcription rates of the $G_{i\alpha2}$ and $G_{i\alpha3}$ genes in control or isoproterenol-treated pregnant rat myometria assessed by nuclear run-on transcription assay. Nuclei were prepared from control (C D21) or 76 h after administration of isoproterenol (I 76 h) in the presence of [^{32}P]UTP. The ^{32}P-mRNAs were isolated and hybridized to $G_{i\alpha2}$ or $G_{i\alpha3}$ subunit cDNA or cyclophilin cDNA immobilized to a nitrocellulose membrane (top panel). Relative $G_{i\alpha2}$ (A) and $G_{i\alpha3}$ (B) transcription rates are expressed as percent of control D21. The data shown are the average ± S.E. of three experiments; [a] indicates a statistically significant difference from control D21 (P<0·05, non-paired Student's t-test).

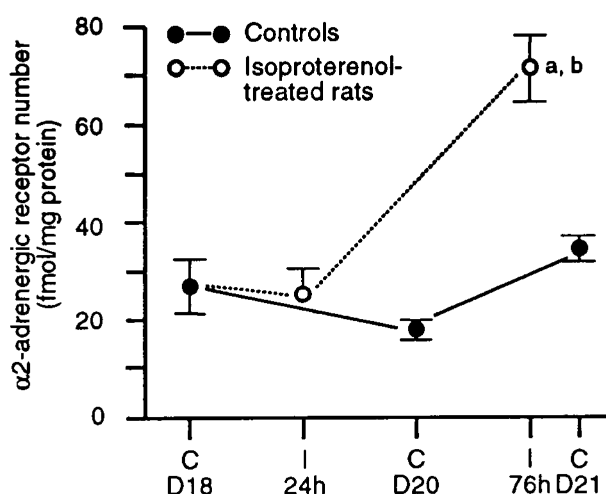

Figure 4 Time course of changes in α_2-AR numbers in plasma membranes of control and isoproterenol-treated rats. Pregnant rats were twice-daily administered isoproterenol (I), from day 18 to day 21 of pregnancy. Control rats at days 18, 20 and 21 of pregnancy (C D18, C D20, C D21) were treated with vehicle. α_2-AR density (B_{max} value) was determined by the binding of [^{3}H]rauwolscine (1–30 nM) and Scatchard plot analysis. The data are the mean values ± S.E. of four to ten experiments; [a] indicates a statistically significant difference from controls of the same stage of pregnancy; [b] indicates a statistically significant difference from previous stage (P<0·05, non-paired Student's t-test).

cascade implicates an obligate role for cAMP/protein kinase A in the up-regulation of $G_{i\alpha2}$ expression. Despite the fact that no data are currently available on the promoter region of G_i genes in the rat, it could be speculated that the mechanisms underlying the increase of $G_{i\alpha2}$ and $G_{i\alpha3}$ subunits reported in this paper also depend on cAMP responsive enhancer elements. cAMP transcriptional regulation of the $G_{i\alpha2}$ gene may also require the induction of a member of the CCAAT-box family of DNA-binding protein, as it has been demonstrated in pig kidney derived LLC-PK cells (Kinane *et al.* 1993).

In HT29 human colonic adenocarcinoma cells, Sakaue & Hoffman (1991) have shown that elevated intracellular cAMP concentrations also increase α_2-AR numbers. Taking these observations into account, we have investigated the effect of repeated injections of isoproterenol on myometrial α_2-ARs which are co-localized with β_2-ARs in longitudinal muscle of myometrium (Legrand *et al.* 1991, 1993). Two α_2-AR subtypes, α_{2A}- and α_{2B}-, have been previously characterized by pharmacological and molecular techniques (Legrand *et al.* 1993, Mhaouty *et al.* 1995). These subtypes show differential expression during the course of pregnancy; at mid-pregnancy, the abundance of the α_{2A}-AR mRNA is maximal. Then it declines, whereas α_{2B}-AR mRNA levels increase in the late stages of pregnancy (Mhaouty *et al.* 1995). The results of the present study showed that repeated injections of isoproterenol between day 18 and day 21 of pregnancy selectively increased the density of the α_{2A}-subtype. By 76 h of isoproterenol treatment, the α_{2A}-AR mRNA levels were 2·5-fold higher than those in control myometria of the same stage of pregnancy (day 21). From the data of Sakaue & Hoffman (1991) in HT29 cells, it seems reasonable to assume that the increase in myometrial α_{2A}-mRNA levels is due to an increased rate of transcription of the α_{2A} gene mediated by cAMP via protein kinase A. This possibility is supported by the identification of cAMP enhancer elements in the promoter region of the human gene (Fraser *et al.* 1989, Shilo *et al.* 1994). A similar regulatory process cannot be discarded in the pregnant rat myometrium. However, studies on the α_{2A} gene promoter in rat are needed to clarify this question.

The comparison of our results with pregnancy-related changes of G_i protein and α_{2A}-AR levels may contribute to a better understanding of the regulation of those two entities. We previously reported a high amount of both G_{i2} and G_{i3} proteins at mid-pregnancy. G_{i2} remained elevated at term under the effect of estrogen, whereas G_{i3} drastically

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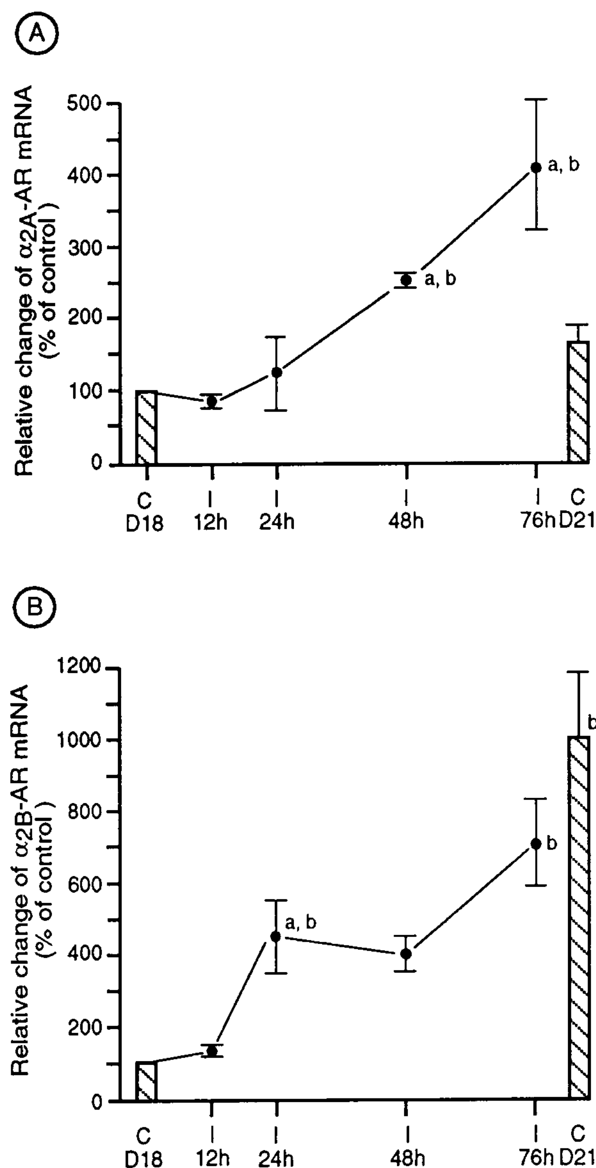

Figure 5 Time course of changes in α_{2A}- and α_{2B}-AR mRNA values in control pregnant myometria or in myometria following administration of isoproterenol. Pregnant rats were exposed twice daily to isoproterenol from day 18 to day 21 of pregnancy. Control rats were treated with 0·9% NaCl (vehicle). mRNA values were determined by Northern blotting using rat α_{2A}- and α_{2B}-AR cDNA probes. A rat cyclophilin probe was used as an internal control. The autoradiograms were scanned using a densitometer. The changes observed in the amount of α_2-AR mRNA subtypes following administration of isoproterenol are expressed relative to control D18. The data are the average ± S.E. of three or four experiments; [a] indicates a statistically significant difference from control on day 18 or 21 of pregnancy; [b] indicates a statistically significant difference from previous stage (P<0·05, non-paired Student's t-test).

decreased. The decrease of both G_{i3} protein and mRNA in the late pregnant rat was triggered neither by the decline of progesterone nor by the increase of estrogen (Cohen-Tannoudji *et al.* 1995). This led us to suggest that G_{i3} expression in the pregnant myometrium was not under the control of steroid hormones. We also observed a predominant expression of α_{2A}-AR subtype at mid-pregnancy (73% vs 27% at term), in agreement with the maximal abundance of their mRNAs (Mhaouty *et al.* 1995). However, we failed to demonstrate until now any direct regulation by steroids hormones. Altogether, our results suggest that the experimental design developed in that study mimics what happens during pregnancy. Indeed, at this period, the G_s protein/adenylyl cyclase/cAMP cascade is strongly activated by various stimulatory agents (catecholamines, relaxin, PGE_2 and I_2). This may contribute to the high expression of myometrial α_{2A}-AR subtype and G_i protein.

One may hypothesize that such cross-regulation between β-AR stimulatory pathway and α_2-AR/G_i inhibitory pathway could contribute to a limited adenylyl cyclase activity, thereby causing an increase in uterine tone or contractions. In this regard, the physiological significance of our *in vivo* observations is not clear, since isoproterenol treatment administered between days 18–20 of pregnancy does not generate myometrial hypercontractility or premature labor (Ikeda *et al.* 1984, our unpublished observations) whereas it does when administered at the peri-partum period (Kocan *et al.* 1993, Herman-Gnjidic *et al.* 1994). This could be related to the strong differences in myometrial steroid environment at term (estrogen dominance), during pregnancy (progesterone dominance) (see Maltier *et al.* 1989) or after isoproterenol treatment in the rat. Indeed, previous studies have shown that β-agonist administration in rat increases ovarian progesterone secretion (Ratner *et al.* 1980, Norjavaara *et al.* 1982). Our hypothesis is supported by the data of Wu *et al.* (1989) demonstrating that progesterone diminishes the inhibitory coupling of α_2-ARs to myometrial adenylyl cyclase whereas, under estrogen treatment, this coupling is raised. This probably explains why β-agonists differently maintain myometrial relaxation depending on the time of pregnancy at which they are administered. Their effectiveness in maintaining myometrial relaxation could be modulated by additional factors controlling the functional state of the receptors/G proteins/adenylyl cyclase machinery.

Altogether, our results give new insights on the physiological relevance of the complexity of cross-regulations in *in vivo* models.

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