0026-895X/88/040389-07$02.00/0

MOLECULAR PHARMACOLOGY, 33:389-395

Estrogen Reduces β-Adrenoceptor-Mediated cAMP Production and the Concentration of the Guanyl Nucleotide-Regulatory Protein, G_s, in Rabbit Myometrium

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Received October 6, 1986; Accepted January 15, 1988

SUMMARY

The uterine contractile response to adrenergic agonists or sympathetic stimulation is influenced dramatically by the hormonal milieu. Rabbit uterine contraction is mediated by α_1-adrenoceptors, whereas relaxation in response to the same stimulus is mediated by β_2-adrenoceptors. Whether uterine contractility is increased or decreased by adrenergic stimulation is determined by the gonadal steroids estrogen and progesterone: uterine contraction prevails in the estrogen-dominant or the ovariectomized animal, but in the progesterone-dominant rabbit, uterine relaxation is observed. In previous studies, we have demonstrated that changes in the concentration or agonist affinity of these adrenoceptors cannot account for the changes in contractile response. In the present studies, we tested whether sex steroids might alter β-adrenergic response by acting on events distal to receptor occupancy, and whether this could explain the conversion of contractile response. We found that myometrial cAMP generation is potently stimulated by β-agonists in progesterone-treated and also in ovariectomized animals, but this stimulation is absent after estrogen treatment. Similar, but smaller, changes were observed for cAMP generation in response to prostaglandin E_2 and forskolin. Stimulation of adenylate cyclase in uterine particulates by agents which act on the guanyl nucleotide-sensitive stimulatory transducer, G_s, is unchanged after estrogen treatment. However, specific labeling of G_s catalyzed by cholera toxin is reduced in membrane particulates from estrogen-treated animals. Recombination of extracts of uterine membranes from the differently treated animals also suggested qualitative differences in G_s. We conclude that at least one component of the adenylate cyclase cascade beyond the β-adrenoceptor, i.e., G_s, is a target for ovarian steroids; estrogen reduces G_s labeling and β-adrenoceptor-mediated cAMP production. However, uterine G_s labeling and cAMP production are similar in ovariectomized and in progesterone-treated rabbits. Since these uteri exhibit different contractile responses, the observed changes are not sufficient to explain sex steroid-mediated conversion of myometrial contractile response.

For nearly a century it has been recognized that the adrenergic contractile response of the uterus is regulated by ovarian steroids. Dale (1) observed that the uterine response to epinephrine was enhanced contractility in the nonpregnant rabbit, but contractility was observed to be inhibited during pregnancy (2). This observation has since been extended to several species (3–5), including humans (6) and has been shown to be a conversion from contraction mediated by α-adrenoceptors to β-adrenergic inhibition of contraction (3). These effects are known to be caused by ovarian steroids, since, in the rabbit, they can be mimicked by treatment with exogenous hormones. Miller and Marshall (4) reported that the uterus from rabbits treated with estrogen responded to hypogastric nerve stimulation by contracting, and that this could be prevented by the α-antagonist phentolamine. However, if the animal was treated with progesterone after estrogen, the response to the same stimulation was inhibition of contractions, a β-adrenergic response. Thus, estrogen dominance results in contraction of the rabbit uterus, whereas progesterone dominance results in relaxation in response to catecholamines.

Our previous work revealed that, although estrogen administration to rabbits markedly increased myometrial α receptor concentration, the conversion from α- to β-adrenergic contractile response with progesterone could not be accounted for by changes in the concentration of β receptors, or the ratio of α to β receptors: α-adrenoceptor concentration is increased by es-

This work was supported by National Institutes of Health Grants HD 16262 and HD 21785. R. K. R. and S. P. B. were supported by the Mellon Foundation. M. M. J. is the recipient of National Institutes of Health Clinical Investigator Award HD00623.

ABBREVIATIONS: G_s, stimulatory guanine nucleotide-regulatory protein; E_2, estradiol; E_2P_4, estrogen/progesterone treatment; Ovx, ovariectomy; IBMX, isobutylmethylxanthine; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; MOPS, 4-morpholineethanesulfonic acid; ANOVA, analysis of variance; PGE_2, prostaglandin E_2; GPP, guanosine 5′-imidodiphosphate.

trogen, but steroid treatment does not change the concentration of uterine β-adrenoceptors (7). Also, the uterus of ovariectomized rabbits, which contracts in response to adrenergic stimuli, has the same concentration of α- and β-adrenoceptors as the noncontractile progesterone-dominant uterus. Since the ability of β agonists to inhibit smooth muscle contraction is mediated by increased intracellular cAMP (8), we asked if hormonal treatment might modify uterine cAMP production. We found that the maximal production of cAMP in uterine minces in response to β-adrenergic stimulation was reduced after estrogen treatment, compared to the response after progesterone treatment or withdrawal of sex steroids by ovariectomy. To try to better understand this effect of estrogen, we examined the effect of treatment on adenylate cyclase components distal to the β-adrenergic receptor. We found that the reduced cAMP response after estrogen treatment was accompanied by a lower concentration of membrane protein substrate for cholera toxin-stimulated ADP ribosylation, which we interpret as a decrease in functional G_s. No other striking differences in uterine adenylate cyclase components were observed among the treatment groups. These studies suggest that G_s is regulated by estrogen, and that these effects may contribute to the decreased β-adrenergic stimulation of uterine adenylate cyclase. They also suggest, however, that the mere ability to produce cAMP is insufficient to explain relaxation with adrenergic stimulation of the progesterone-dominant uterus, since the ovariectomized rabbit uterus can also produce cAMP yet adrenergic stimuli cause contraction.

Materials and Methods

Drugs and materials. [^{3}H]cAMP was from New England Nuclear. γ-[^{32}P]ATP was from Amersham. All other agonists and hormones were obtained from Sigma Chemical Co.

Treatment. Mature female New Zealand White rabbits were ovariectomized bilaterally under ketamine/xylazine anesthesia. Seven or more days after surgery, estradiol benzoate in sesame oil vehicle was administered for four successive days at a dose of 50 μg/kg, intramuscularly (E_2). Animals treated with estrogen and progesterone (E_2P_4) were first treated with the same estrogen dosage for 4 days, and then given progesterone in sesame oil vehicle at a dose of 5 mg/kg, intramuscularly, for the next 4 days. Other rabbits were treated with vehicle instead of estrogen for 4 days (Ovx). The rabbits were sacrificed 24 hr after the last steroid or vehicle dose.

cAMP generation and assay. cAMP generation was performed with 1 × 1 mm cubes of myometrium prepared with a McIlwain tissue chopper (Brinkmann Instruments). After mincing, the explants were incubated 1 hr in aerated (95% O_2, 5% CO_2) Krebs buffer (pH 7.4), at 25°. They were then washed, and aliquots, 20–40 mg (wet weight), were incubated in 5 mM Tris (pH 7.4), 0.15 M NaCl, containing 1 mM IBMX at 37° for 5 min in the presence or absence of effectors. The reaction was stopped by adding sufficient trichloroacetic acid to result in a 5% solution. Samples were stored for 12 hr at 4°, then the trichloroacetic acid was removed by ether extraction. We found that the initial aeration reduced cAMP generation in the absence of agonist, perhaps by allowing the metabolism of endogenous prostanoids generated by tissue preparation. cAMP generation was linear with time and tissue weight under the conditions of the assay. cAMP was measured as described by Gilman (9). All the material measured as cAMP was degraded by cAMP phosphodiesterase.

Adenylate cyclase. Adenylate cyclase activity was determined by a modification of the Salomon technique (10) on particulates prepared as described previously (7). Protein concentration was determined by the method of Bradford (11) with bovine serum albumin as standard. The methods used for the extraction and recombination of G_s from uterine membranes into S49 cyc$^-$ cell membranes were essentially as described by Bokoch (12). The reaction mix for the adenylate cyclase assay containing S49 cyc$^-$ cell membranes (20 μg of protein), 10 μl of Lubrol-extracted uterine G_s proteins, and any effectors, were incubated for 20 min at 30° prior to the addition of [^{32}P]ATP to start the adenylate cyclase activity.

[^{32}P]ADP ribosylation of G_s with cholera toxin. ADP ribosylation was conducted as described by Johnson *et al.* (13). Cholera enterotoxin was activated by incubation of toxin (1 mg/ml) in 20 mM dithiothreitol for 30 min at 37°. For ribosylation, the membrane particulate (0.25 mg/ml of protein) was incubated with the activated toxin for 30 min in 250 mM K_2HPO_4/KH_2PO_4 (pH 6.6), 12 mM thymidine, 1 mM ATP, 0.1 mM GTP, 1 mM $MgCl_2$, and 10 μCi of [^{32}P]NAD$^+$ at 30°. Membranes were collected by centrifugation at 30,000 × g for 20 min and washed twice with 50 mM Tris-Cl (pH 7.5). The membrane pellets were resuspended in SDS-PAGE sample buffer (20% glycerol, 10% 2-mercaptoethanol, 4% SDS, 0.05% bromphenol blue, and 125 mM Tris-Cl, pH 6.8), then heated at 60° for 1 hour. The samples were then loaded on an SDS slab gel (14 cm × 10 cm × 1 mm, 12% acrylamide, 0.32% methylbisacrylamide, and a 3% acrylamide, 0.08% methylbisacrylamide stacking gel). The tank buffer contained 192 mM glycine, 25 mM Tris-Cl (pH 8.3), and 0.1% SDS. The gels were run at a constant current of 5–7 mamp/gel. ADP ribosylation was examined by autoradiography (10–20 hr exposure at −70°) using Kodak X-Omat AR film developed in a standard automated X-ray film processor, and scanned by laser densitometry (Zeineh Soft Laser, Biomedical Instruments, Chicago, IL). Identical samples were incubated without cholera toxin to allow determination of toxin-independent ADP ribosylation.

ATP determination. Tissue ATP content was measured as described by Helgerson *et al.* (14). Samples of tissue between 35 and 45 mg were weighed and ground in liquid nitrogen using a mortar and pestle. The tissue was recovered, placed in a tared tube, thawed, and reweighed to determine recovered tissue weight. Next, 250 μl of 0.42 M perchloric acid were added to each sample. A magnetic stir bar was added to each tube, and the samples were extracted on ice for 10 min on a magnetic stirrer (high speed). The stir bars were removed and the tubes were spun in a microcentrifuge for 3 min to pellet the tissue. Supernatant (200 μl) was taken from each tube and placed in a separate tube, on ice, until the next step. The pellet was resuspended in 250 μl of perchloric acid with a pipette, the stir bars were added, and a second extraction was done for 10 min on ice with stirring. The tubes were spun again in a microcentrifuge for 3 min and a second aliquot of 200 μl of the supernatant was pooled with the first. Next, a sufficient volume (~100 μl) of imidazole (6 M) was added to the pooled extracts to bring the pH to neutrality. Extracts were frozen in liquid nitrogen and stored at −70° awaiting the Luciferin enzyme assay. ATP was quantified using the Luciferin-Luciferase assay as described by Kimmich *et al.* (15). A DuPont Luminesence Biometer was used to quantify the light produced by the reaction. A standard curve was generated by injecting either 3 or 10 μl of ATP (in 10 mM MOPS, 5 mM $MgSO_4$, pH 7.9) in concentrations ranging from 1 ng/ml to 100 μg/ml. Unknowns were injected into the Biometer in 10-μl quantities. The ATP concentration was found by comparing extract photons against a linear regression (log-log plot) of the standard curve. The ATP concentration was then corrected for tissue weight and assay volume.

Data analysis. Data are presented as the mean and standard error. For cAMP generation, the data are expressed as multiples of cAMP generation without effector (basal cAMP generation) in the same preparation. Basal generation was not changed by treatment [E_2, 8.2 ± 2.1 (n = 6); E_2P_4, 6.1 ± 1.8 (n = 6); Ovx, 6.4 ± 0.4 (n = 4), pmol/mg wet weight/5 min; mean ± SE). Comparison of the data was by one-way ANOVA for isoproterenol response and ATP content, and two-way ANOVA for comparisons of the effect of hormonal treatment on the response to other effectors. Statistical significance was accepted at $p < 0.05$, in a one-tailed test, since our hypothesis was that estrogen treatment would be associated with decreased G_s activity.

Results

cAMP generation. Isoproterenol caused a dose-dependent increase in cAMP generation in uterine minces from Ovx- and E_2P_4-treated rabbits. The dose of isoproterenol causing half-maximal response (EC_{50}), which was 0.2 μM, was not different in either group and was similar to that reported for isoproterenol in rat myometrium (16). By contrast, isoproterenol did not increase cAMP generation in myometrium from E_2-treated rabbits (Fig. 1). To determine whether the hormonally related changes in cAMP response were unique to β-adrenergic stimulation, we compared cAMP generation stimulated by PGE_2 in the differently treated animals (Fig. 2). In contrast to isoproterenol, PGE_2 stimulated the generation of cAMP in myometrium from estrogen-treated animals (Fig. 2A), but there was still significantly more stimulation in E_2P_4 and Ovx tissue. In an attempt to activate adenylate cyclase in uterine minces independent of receptor occupancy, we examined cAMP generation in response to forskolin, which is thought to directly stimulate G_s and/or the catalytic component of adenylate cyclase (and can act on intact cells). As with PGE_2, forskolin increased cAMP in myometrium from estrogen-treated rabbits, but to a lesser degree than in Ovx or E_2P_4 animals (Fig. 2B).

Uterine ATP content. Since reduced cAMP generation in E_2-treated animals was demonstrated in intact tissues, we asked whether a difference in ATP as substrate might explain the reduced cAMP generation. Measurements of uterine ATP content showed no difference ($p = 0.11$ by one-way ANOVA) in the amount of ATP per gram of tissue wet weight among the treatment groups (Table 1).

Adenylate cyclase activity in uterine membranes. In spite of numerous perturbations (magnesium and GTP concentration, nonhydrolyzable ATP analogues, etc.), we have not been able to demonstrate β agonist-stimulated adenylate cyclase activity in particulate preparations of rabbit myometrium. This problem has been reported for other tissues and may reflect the loss or degradation of a critical cyclase component(s). However, we were able to use particulate preparations to examine other components of the adenylate cyclase system using effectors acting beyond the receptor. The EC_{50} for GTP (Fig. 3A) and for GPP (Fig. 3B), was similar in both groups. Examination of GTP, GPP, NaF, Mn^{2+}, and forskolin stimulation in the two preparations indicated no significant differences in adenylate cyclase activation by these effectors (Fig. 4).

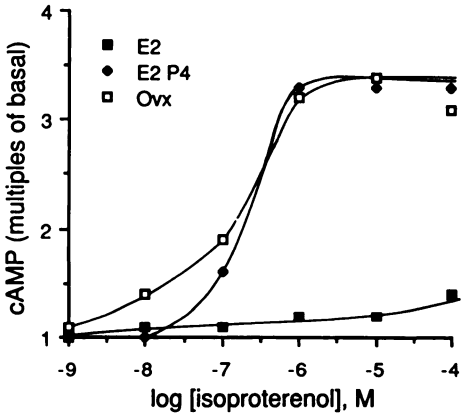

Fig. 1. Isoproterenol stimulation of cAMP generation. Myometrial minces from the differently treated animals were incubated with isoproterenol at increasing concentrations for 5 min at 37° in the presence of 0.1 mM IBMX. cAMP was measured as described under Materials and Methods. Data are the mean of four experiments for each treatment, determined in triplicate. Stimulation of E_2 samples by isoproterenol (0.1 mM) was not significantly greater than basal, and stimulation was greater than E_2 for all doses of isoproterenol greater than 10^{-7} M in Ovx and E_2P_4 samples.

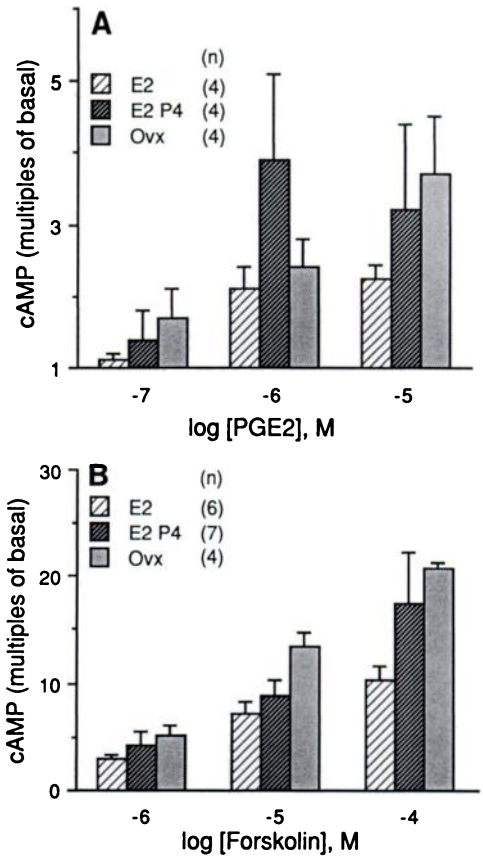

Fig. 2. Stimulation of cAMP generation by PGE_2 (A) and forskolin (B). Myometrial minces from the differently treated animals were incubated with the indicated concentrations of forskolin and PGE_2 for 5 min at 37° in the presence of 0.1 mM IBMX. cAMP was measured as described under Materials and Methods. Data are the mean and standard error of the indicated number of experiments determined in triplicate. Two-way ANOVA indicated that, for E_2, the generation of cAMP was less than in either of the other groups ($p < 0.05$), which were not different. The responses to individual doses were not significantly different.

TABLE 1
Uterine ATP content

Treatment	(n)	ATP content*
		ng/mg wet weight
E_2	(4)	175 ± 62
E_2P_4	(4)	237 ± 31
Ovx	(4)	93 ± 25

* Values are means ± standard errors.

Recombination of uterine G_s with S49 cyc$^-$ membranes. The cyc$^-$ variants of the S49 lymphoma cell line do not express G_s activity but contain β-adrenoceptors and the catalytic component of adenylate cyclase. Thus, these cells can be used to test the expression of functional G_s from other tissues. We recombined G_s extracted from uterine membranes obtained from E_2- and from E_2P_4-treated rabbits with cyc$^-$ cell membranes to determine whether G_s expression was altered by hormonal treatment in a manner which would be apparent in this system. The data in Fig. 5a show the results of these studies. Recombination enabled the expression of agonist sen-

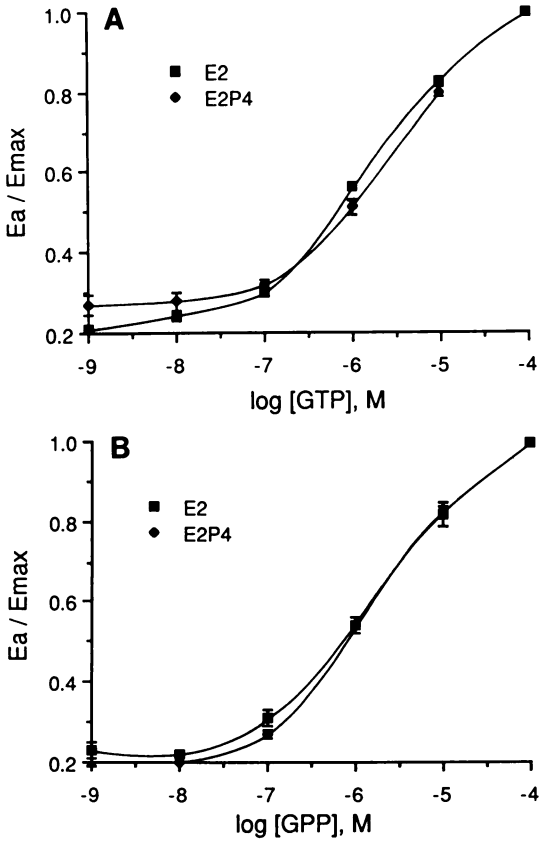

Fig. 3. Relative dose response to GTP (A) and GPP (B) in particulate preparations of rabbit myometrium. Data are the mean and standard error (if error bars are not seen they were smaller than the symbols) of triplicate determinations of response (E_a) as a fraction of maximal response (E_{max}) in four to five experiments. Response to 1 nM GTP and GPP was not different from basal levels, with either treatment. Basal adenylate cyclase activity and maximal response to the effectors are illustrated in Fig. 4.

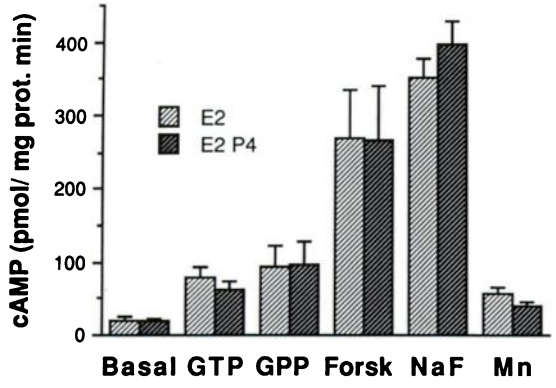

Fig. 4. Adenylate cyclase activation in rabbit myometrial particulate. Data are presented as the mean and standard error of triplicate determinations in eight to nine experiments (except $n = 4$ for Mn^{2+}). Shown are the responses to maximally effective concentrations of GTP, GPP, forskolin (all 0.1 mM), and NaF and Mn^{2+} (both 10 mM). The responses to individual effectors were not significantly different with the two treatments.

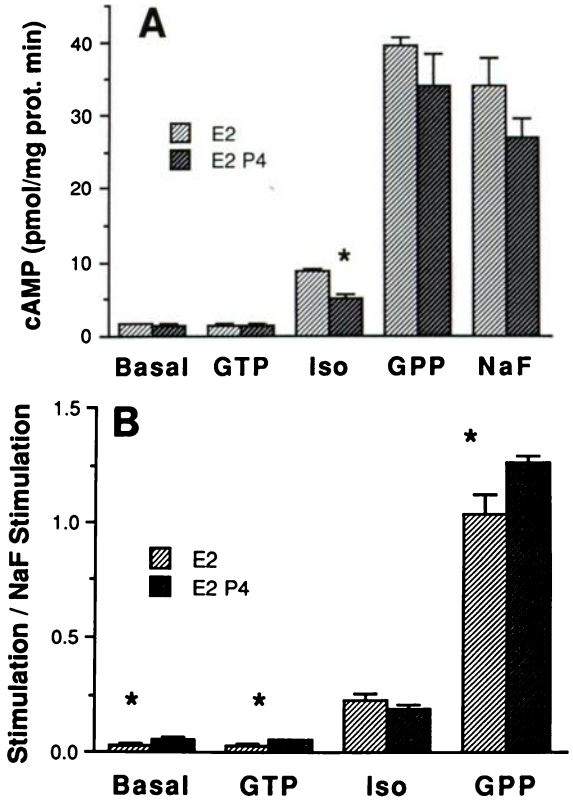

Fig. 5. Expression of uterine G_s in S49 cyc^- cell membranes. G_s was extracted from uterine membranes obtained from differently treated rabbits and recombined with cyc^- membranes. Adenylate cyclase activity in response to various effectors was then determined as described under Materials and Methods. A shows the cAMP production (mean ± standard error) from four uteri from each treatment group. The activity stimulated by isoproterenol (10 μM) in the presence of GTP (100 μM) was significantly reduced in the recombinations with G_s from E_2P_4 rabbits ($p = 0.01$ by unpaired t test corrected for multiple comparisons). The other effectors were used at a concentration of 100 μM (GTP and GPP) and 10 mM (NaF). B shows the data in A, after normalization to the NaF response.

sitivity which could not otherwise be demonstrated in the native uterine preparations. In the recombined system, the only significant difference observed between G_s from the differently treated uteri was a decreased ability to stimulate with isoproterenol in the E_2P_4 membranes. The fact that mean values for the stimulation of adenylate cyclase by NaF, an agent which directly stimulates the α subunit of G_s, were higher for E_2 treatment in the recombination experiments and lower in native membranes (Fig. 4) suggested a differential efficiency of recombination in the two treatment groups. In an attempt to control for the efficiency of recombination, we also examined data normalized to the response to NaF. With this normalization, GTP and GPP stimulation of cyclase activity was significantly greater in extracts frfom E_2P_4 rabbits, whereas isoproterenol stimulation was not different (Fig. 5b).

Cholera toxin-stimulated ADP ribosylation. In order to determine whether decreased adenylate cyclase activation with estrogen treatment might be accounted for by a decrease in the relative amount of guanyl nucleotide-sensitive coupling protein, G_s, we measured the cholera toxin-catalyzed incorporation of [^{32}P]NAD into myometrial membranes from the differently treated animals. Examination of autoradiograms of SDS-PAGE gels demonstrated cholera toxin-specific labeling of a 44-kDa singlet and a 47-kDa doublet in all groups (Fig. 6). However,

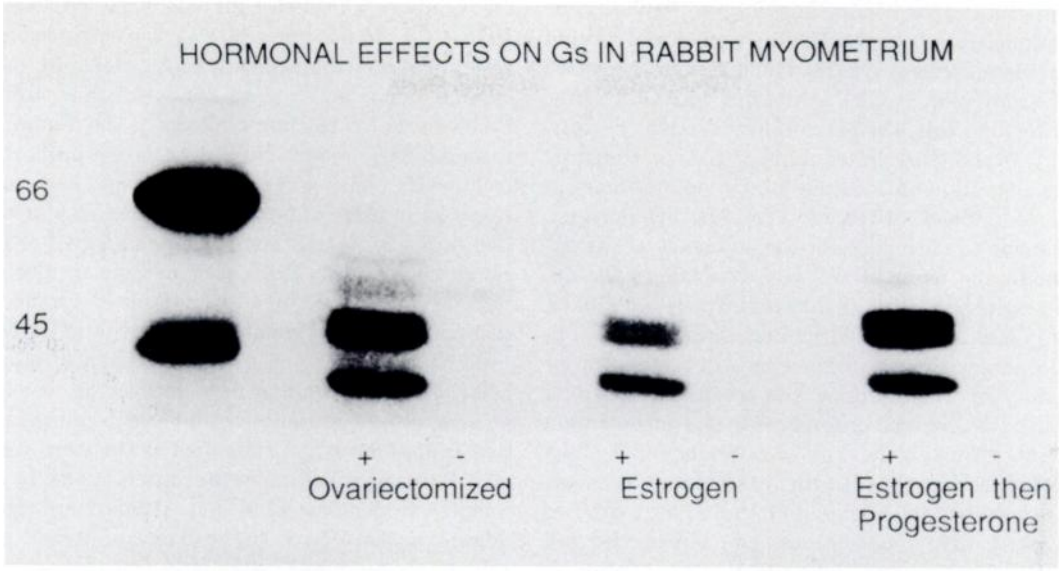

Fig. 6. Effect of steroid treatment on myometrial G_s labeling. A representative autoradiogram of [^{32}P]ADP-labeled proteins from rabbits treated as indicated is shown. ADP ribosylation with [^{32}P]NAD was performed with (+) or without (−) cholera toxin. Iodinated molecular weight standards in the *left* lane are 66,000 (upper band) and 45,000 (lower band) of this SDS-PAGE gel.

there was a reduction in the density of these proteins in preparations from E_2 animals (Table 2) compared with preparations from Ovx and E_2P_4 animals. In all preparations, the protein concentration used for the labeling studies (0.5 mg/ml) was within the range over which labeling increased in proportion to increased membrane particulate.

Discussion

Our studies indicate that ovarian steroids modify β-adrenoceptor activation of adenylate cyclase in uterine tissue. Associated with this is a reduction in the labeling of specific cholera toxin substrates and changes in some, but not all, G_s-mediated functions. The β agonist isoproterenol minimally stimulates cAMP generation in E_2 rabbits compared to E_2P_4 and Ovx animals in spite of similar β receptor concentration (7). In addition, an agent which acts at a different receptor, PGE_2, and an agent acting beyond receptors, forskolin, are also slightly less potent in uteri from E_2 animals. Because these studies were performed in intact tissue, our findings could be explained by decreased activation of adenylate cyclase, an increase in phosphodiesterase activity leading to increased degradation of cAMP, or a reduction of ATP substrate in the estrogen-treated myometrium. Since the experiments were performed with what we have determined to be an optimally effective concentration of the phosphodiesterase inhibitor IBMX, we feel the second hypothesis is an unlikely explanation. Measurements of ATP content did not reveal differences. Thus, it seems likely that the decreased uterine cAMP production after estrogen treatment is actually due to decreased adenylate cyclase activation.

TABLE 2

Cholera toxin-catalyzed ADP-ribosylation of rabbit myometrial membranes: scanning densitometry data from autoradiograms

Data are presented as arbitrary units of integrated area (mean ± standard error). Data were compared by paired *t* test (corrected for multiple comparison) of densities of bands in autoradiograms from the same gel (one gel did not contain an Ovx sample). Values for E_2P_4 and Ovx were significantly greater ($p < 0.05$) than E_2 values for both bands and total density, but were not different from each other.

Treatment	(*n*)	Relative density of cholera toxin-specific bands		
		44 kDa	47 kDa	Total
E_2P_4	(4)	0.48 ± 0.12	0.93 ± 0.18	1.31 ± 0.26
Ovx	(3)	0.45 ± 0.12	0.71 ± 0.16	1.16 ± 0.28
E_2	(4)	0.19 ± 0.06	0.30 ± 0.09	0.50 ± 0.12

Cholera toxin-specific substrates for ADP ribosylation are present in rabbit myometrium and their relative abundance is reduced after estrogen treatment compared with Ovx and E_2P_4 rabbits. The substrates include not only the 44-kDa substrate present in the pigeon erythrocyte (17), but also a higher molecular weight doublet (47 kDa), which is also present in reticulocytes (18) and preadipocytes (19). It is possible that the higher molecular weight substrate is present in tissues undergoing rapid changes in function, such as hormonal targets or predifferentiated tissue. Although the labeling of both the 44-kDa and 47-kDa doublet was less in myometrium of estrogen-treated animals, the decrease was greater in the 47-kDa bands. It is likely that the reduction in the ADP ribosylation of G_s indicates a reduced concentration of this protein. Alternative possibilities, such as a decrease in labeling sites on G_s, or increased endogenous ADP ribosylation with estrogen treatment, seem less likely but are difficult to exclude. Increased NADase activity stimulated by estrogen also does not explain the reduced labeling because labeling increased proportionately with membrane protein, which indicates that NAD concentration was not limiting the ADP ribosylation reaction. The similar cAMP generation and G_s labeling in the Ovx and E_2P_4 animals indicates that the reduction in β-adrenergic activation of adenylate cyclase and G_s labeling is secondary to estrogen treatment, rather than a progesterone effect.

Recombination of extracts containing uterine G_s into cyc^- cell membranes revealed qualitative changes in G_s with treatment. With recombination into cyc^- cells, G_s extracts from E_2- as well as E_2P_4-treated uterus supported isoproterenol-stimulated adenylate cyclase activity which could not otherwise be

demonstrated in the native uterine membrane preparations. Another striking contrast between G_s function in native and cyc^- recombined membranes is a marked increase in the relative effectiveness of stimulation by GPP, compared with GTP, after recombination. Beyond this, the recombination data are difficult to interpret. If the data are examined without transformation, there is actually more stimulation by isoproterenol in the membranes recombined with extracts from E_2 myometrium. If attempts are made to normalize for the efficiency of extraction and recombination using stimulation by NaF as the denominator, the results appear quite different: the activity of G_s from E_2 uterus is less, as indicated by decreased basal, GTP, and GPP stimulation, with no difference in isoproterenol stimulation. Normalized in this manner, the recombination data are consistent with the observed differences in G_s concentration with E_2 treatment, although the lack of difference in agonist response is clearly inconsistent with the intact tissue response. (Similar normalization of the adenylate cyclase data obtained in myometrial particulate preparations, i.e., Fig. 4, did not change the results). Regardless of how the data are expressed, these findings indicate qualitative differences in G_s with treatment which are apparent upon insertion into cyc^- acceptor membranes: catecholamine response is restored and differences in sensitivity to guanyl nucleotides are revealed. This suggests either the presence of components in the native membranes which inhibit agonist activation or components present in cyc^- membranes which restore the defect in native membranes. Recent investigations suggesting the importance of the stoichiometry between G_s α and $\beta\gamma$ subunits to cyclase function might be pertinent to these findings. The differential sensitivity to guanyl nucleotides may also result from the apparent differences in the concentration of Gs α isoforms in the two treatment groups (see Fig. 6).

It does not appear that the difference in G_s with treatment entirely explains the reduced cAMP response to β receptor occupancy in E_2 rabbits. The differences in forskolin and PGE_2 response in intact tissue were modest compared with the markedly altered response to isoproterenol in myometrial minces. In addition, if adenylate cyclase stimulation is examined in a cell-free system, there are no apparent differences in adenylate cyclase activation by effectors acting at G_s or beyond. One possible explanation would be that the amount of the catalytic moiety of adenylate cyclase is limiting in this system, and that increases in G_s would primarily affect the interaction of the receptor and G_s. In such a setting, stimulation of G_s by maximally effective concentrations of agents which directly activate this protein would not be different, although the dose-response relation for such agents would be expected to indicate an increased sensitivity with increased G_s. However, the results of our studies of G_s activation by GTP or GPP do not support this hypothesis, as the relative dose responses were identical in myometrial particulates from E_2 and E_2P_4 rabbits. Furthermore, we have previously shown that β-adrenoceptor-G_s coupling, assessed by the ability to form a GTP-sensitive high affinity binding complex, is not different between preparations from E_2 and E_2P_4 rabbits (20). Thus, the change in the uterine β adrenergic system which results in marked differences in cAMP response in intact tissue appears more subtle at the subcellular level.

The basis for the lack of striking changes in G_s function with hormonal treatment may reside in the fact that other receptors use G_s as a transducer for adenylate cyclase activation (eg. PGE_2). We do not know how G_s is partitioned among different receptors, nor the stoichiometry of G_s in relation to total cellular adenylate cyclase catalytic units, and total cellular cyclase-coupled receptors. Hence, if the β-adrenergic receptor-associated G_s accounts for only a minor proportion of the total cellular G_s, changes measured at the G_s level may be obscured by an abundance of non-E_2-sensitive (i.e., unchanged) G_s complexes. Since E_2 treatment results in a greater change in adrenergic compared to PGE_2 response, our results suggest that the population of G_s coupled to uterine β-adrenoceptors may be distinct from the population coupled to PGE_2 receptors.

The nature of "progesterone dominance" of uterine contractility, i.e., the switching from contractile to relaxant response to sympathetic stimulation, under the influence of progesterone, is apparently not explained by the mere ability to generate cAMP through β-adrenoceptor interactions. In previous experiments, we demonstrated that, although estrogen increases α-adrenergic sensitivity, the response of uterine strips from Ovx rabbits to norepinephrine is a contraction mediated by α-adrenergic receptors (7). Our findings in the present study, of similar cAMP generation and G_s ADP ribosylation in Ovx and E_2P_4 animals, do not support the hypothesis that estrogen treatment is associated with α-adrenergic response solely because of its effect on G_s. Thus, the ability of β receptor activation to increase intracellular cAMP is not sufficient to guarantee β-adrenergic-mediated inhibition of contractile response. Under physiologic conditions, the net cAMP generation in the uterus by the endogenous agonists epinephrine and norepinephrine is due not only to the activation of adenylate cyclase through the β receptor, but also the inhibition of adenylate cyclase through interaction with myometrial α_2 receptors. Since α_2-adrenoceptors are present in abundance in the myometrium, and their concentration is increased by estrogen (7, 21), this component of adrenergic response must also be considered. In addition to this possible influence of α_2-adrenoceptor activation on cAMP metabolism, modification of other steps in the response cascade (e.g., kinases and their substrates, Ca^{2+} mobilization) may also contribute to the conversion of contractile responses.

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