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Hormonal Interactions in the Uterus: Inhibition of Isoproterenol-Induced Accumulation of Adenosine 3′:5′-Cyclic Monophosphate by Oxytocin and Prostaglandins

(β-adrenergic effector/rats/adenylate cyclase/uterine contraction)

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ABSTRACT **Interactions of hormones stimulating and inhibiting uterine contraction were studied *in vitro* in uteri from oophorectomized rats. The β-adrenergic effector, isoproterenol, a potent inhibitor of contraction, produced a dose-related increase of adenylate cyclase and accumulation of adenosine 3′:5′-cyclic monophosphate (cAMP) that was inhibitable by propranolol. Oxytocin, which stimulates contraction, effectively inhibited accumulation of uterine cAMP induced by isoproterenol in the presence or absence of theophylline. Prostaglandins E_2 and $F_{2\alpha}$, each at a maximum effective concentration of 0.5 μM, also inhibited accumulation of cAMP induced by isoproterenol, consistent with their effect in stimulation of uterine contraction. Prostaglandin E_2, but not prostaglandin $F_{2\alpha}$, stimulated cAMP accumulation in a dose-related manner at concentrations in excess of 0.5 μM. Neither propranolol nor oxytocin inhibited that response. Bovine endometrial adenylate cyclase failed to respond to isoproterenol but was stimulated by prostaglandins E_1 and E_2. When myometrial preparations were studied, isoproterenol stimulation and prostaglandin effects were observed as for whole castrate uterus. The competitive physiological actions of β-adrenergic effectors on the one hand, and oxytocin and prostaglandins on the other hand, are based on their influences on a myometrial adenylate cyclase. Stimulation of uterine cAMP accumulation by prostaglandin E_2 is due to action at a different and unrelated site.**

Uterine tissue is designed to respond specifically to hormonal influences in order to provide the appropriate environment for implantation, fetal growth and development, and parturition. It appears to be ideal for study of the relationship between steroid-mediated and cAMP-mediated responses. Recently, it was shown that smooth muscle, particularly uterus, has an adenylate cyclase system whose activation by β-adrenergic effectors resulted in inhibition of contractility (1–4). It was further demonstrated that contractions produced by oxytocin, acetylcholine, and calcium were inhibited by β-adrenergic effectors, theophylline, and dibutyryl-cAMP (3, 4) in association with an increase in adenylate cyclase activity (5–7). Prostaglandins, particularly those of the E series, are potent stimulators of uterine contraction despite the fact that they stimulate uterine adenylate cyclase activity (7, 8).

In these studies, we show that the stimulatory effects of oxytocin and prostaglandins E_2 and $F_{2\alpha}$ on castrate uterus and myometrium may well be due to inhibition of cAMP accumulation due to β-adrenergic activity.

Abbreviation; PG, prostaglandin.

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MATERIALS AND METHODS

[^{3}H]cAMP (16.3 Ci/mmol) was obtained from Schwarz BioResearch, isoproterenol was from Winthrop Laboratories, and oxytocin "Pitocin" was from Parke Davis. Prostaglandin E_2 and prostaglandin $F_{2\alpha}$ were generous gifts of Dr. John Pike, the Upjohn Co. Propranolol was obtained from Ayerst Labs, and diethylstilbesterol was from Dome Laboratories. Eagle's minimal essential medium was obtained from Grand Island Biological Co., N.Y.

Female Sprague–Dawley rats, 150–175 g body weight, were ovariectomized 5–8 days before removal of their uteri.

Isolated uterine horns were lightly stretched and tied on glass mounts (9) to give more reproducible results and facilitate movement from one treatment solution to another. The mounted uteri were first incubated in 4 ml of minimal essential medium at 37° for 20 min. They were then incubated according to the experimental protocol. The duration of incubation for all the treatment groups was the same.

At termination, the uteri were quick frozen in liquid N_2, pulverized in a mortar and pestle, and homogenized in 6% Cl_3CCOOH containing 0.38 nM [^{3}H]cAMP in a Polytron tissue disintegrator (Brinkmann). After centrifugation at 1500 × *g* for 20 min the supernatant was collected for the assay of cAMP; the precipitate was used for protein measurement.

Assay of cAMP was by our modification of the method of Walton and Garren (10, 11†), wherein the Cl_3CCOOH supernatant is washed with ether and lyophilized before assay. It is important that the standards be made up and diluted in 50 mM acetate–1 mM EDTA pH 5 buffer containing the nonvolatile contaminants found in the ether-washed Cl_3CCOOH fraction to obtain reproducible results. The details of the assay procedure are given elsewhere.†

RESULTS AND DISCUSSION

Isoproterenol produced a marked increase of uterine cAMP concentration in the presence or absence of theophylline that

† Sanborn, B. M., Bhalla, R. C. & Korenman, S. G. (1973) "Use of a modified radioligand assay to measure the effect of estradiol on uterine adenosine 3′:5′-cyclic monophosphate," *Endocrinology*, in press.

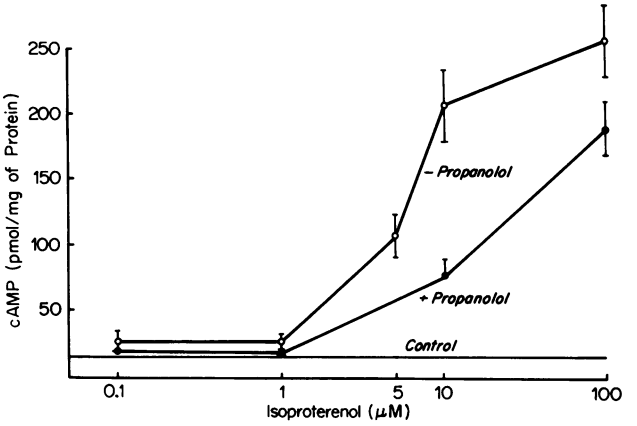

FIG. 1. The dose–response relationship of uterine cAMP to isoproterenol. Incubation was in 5 μM propranolol or control medium for 10 min, followed by isoproterenol for 5 min. All tubes contained 5 mM theophylline. Values are means ±SEM of three separate experiments.

was time- and dose-related and that was inhibited by propranolol (Fig. 1). Half-maximal stimulation was usually obtained at a concentration of about 5 μM with a plateau of peak action at from 5–15 min of incubation.

Oxytocin had no effect on baseline concentration of uterine cAMP in the presence or absence of theophylline. As shown in Table 1, oxytocin produced a dose-related inhibition of isoproterenol stimulation reaching a maximum of about 50%. The order of addition of reagents did not affect the inhibition. Theophylline in concentrations up to 10 mM did not reduce the inhibitory effect of oxytocin, suggesting that phosphodiesterase stimulation was not its mode of inhibition.

By contrast, as seen in Fig. 2, oxytocin failed to alter either basal or isoproterenol-stimulated adenylate cyclase activity through a wide concentration range, confirming a previous report (5). Inhibition of uterine cAMP accumulation was consistent with oxytocin action in stimulating con-

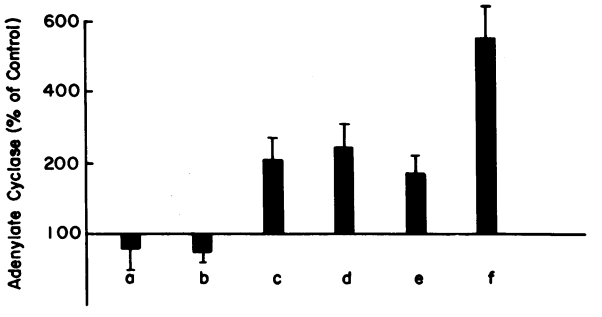

FIG. 2. Effect of isoproterenol and oxytocin on uterine adenylate cyclase. Minced uteri were homogenized in a loose-fitting Dounce homogenizer in 8 volumes of 50 mM Tris·HCl buffer (pH 7.4) containing 1.3 mM 2-mercaptoethanol, 1 mM EGTA, and 6.5 mM $MgCl_2$. The homogenate was filtered through cheese cloth and centrifuged at 1600 × g for 10 min. The pellet was suspended in 50 mM Tris·HCl buffer and recentrifuged. Adenylate cyclase activity was measured by the method of Krishna (12). Values expressed in terms of the activity of controls are means ±SEM, based on four experiments. (*a*) 10 mU of oxytocin/ml; (*b*) 5 mU of oxytocin/ml; (*c*) 10 μM isoproterenol; (*d*) 10 μM isoproterenol + 10 mU of oxytocin/ml; (*e*) 10 μM isoproterenol + 1 mU of oxytocin/ml; (*f*) 0.13 M NaF.

TABLE 1. *Effect of oxytocin on accumulation of isoproterenol-induced cAMP in the absence of theophylline*

Treatment	cAMP (pmol/mg of protein) Exp. 1	Exp. 2	Exp. 3
Control	7.9 ± 3.8	11.0 ± 3.4	3.7 ± 0.02
Isoproterenol	60.1 ± 3.3	102.4 ± 23.1	73.6 ± 7.5
+ oxytocin 1 mU/ml	43.4 ± 6.2	—	—
+ oxytocin 10 mU/ml	30.2 ± 0.8*	40.8 ± 1.7†	47.9 ± 1.3† [32.3 ± 3.8]^{B*}
+ oxytocin 50 mU/ml	34.6 ± 1.6*	—	—

Incubation conditions were the same as in Fig. 1. The duration of treatment with oxytocin was 10 min followed by 5 min with oxytocin + isoproterenol(10 μM). In Exp, 3*B*, the order of incubation was reversed. Values are means of four determinations ±SEM. * $P < 0.01$; † $P < 0.05$; —, not done.

traction of the uterus and in demonstrating competition with β-adrenergic effectors (4, 7). However, in the only other report where specific measurements have been made (13), a significant inhibition was not found. This may have been due to differences in experimental conditions, since in that report estrogen-stimulated uteri were exposed to oxytocin for a very brief period.

Prostaglandins (PG) of the E and F series have been known to stimulate uterine contraction *in vivo* (8). In the one available report of *in vitro* studies, PG E_1 and E_2 caused an increase in uterine adenylate cyclase activity, while $PGF_{2\alpha}$ was inactive (7).

The effects of $PGF_{2\alpha}$ and PGE_2 on uterine cAMP concentration are shown in Fig. 3, which is a composite of four experiments. In each experiment, the amount of cAMP produced by treatment with isoproterenol alone is represented as 100%. $PGF_{2\alpha}$ failed to induce an increase in the amount of uterine cAMP, but PGE_2 was quite active, with a significant increment noted at a concentration of 0.5 μM. However, both PGE_2 and $PGF_{2\alpha}$ were potent inhibitors of isoproterenol stimulation of uterine cAMP with a maximal inhibition achieved at a concentration of 0.5 μM each. At each dose in excess of 0.5 μM, isoproterenol augmented the expected stimulation of cAMP accumulation due to PGE_2. When increasing concentrations of isoproterenol were tested for the effect on uterine cAMP accumulation in the presence or absence of 2 μM PGE_2, inhibition was first noted when a potent

TABLE 2. *Effect of prostaglandins E_2 and E_1 and isoproterenol on cAMP accumulation in endometrium*

Treatment	cAMP (pmol/mg of protein)
Eagle's medium	9.3 ± 1.1
Isoproterenol 10 μM	4.9 ± 1.3
PGE_2 50 μM	25.3 ± 2.9
PGE_1 50 μM	55.4 ± 2.5

Bovine endometrial strips were incubated for 10 min in the presence of 1 mM theophylline. Values represent the mean of three determinations ±SEM.

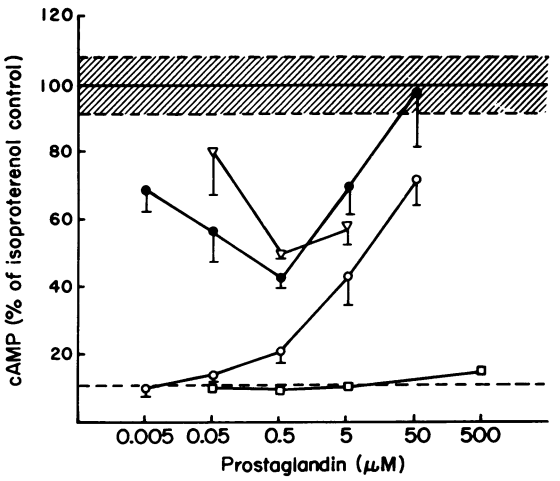

FIG. 3. Effect of prostaglandins E_2 and $F_{2\alpha}$ on accumulation of uterine cAMP induced by isoproterenol. Incubation was for 10 min, in 1 or 5 mM theophylline. Each point is the mean ±SEM of 4–11 individual determinations from four different experiments, expressed as a percent of the isoproterenol control. ▽—▽, $PGF_{2\alpha}$ + isoproterenol; ●—●, PGE_2 + isoproterenol; ○—○, PGE_2; □—□, $PGF_{2\alpha}$; – – –, control; *shaded area*, isoproterenol control (10 μM).

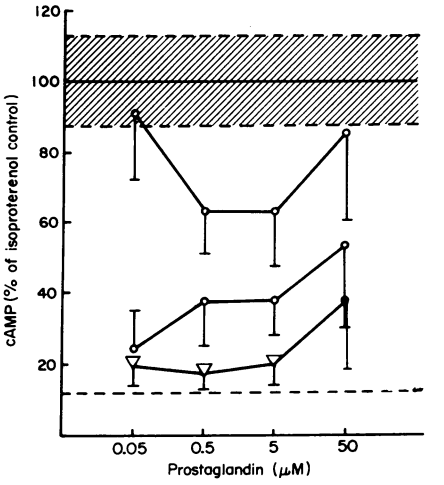

FIG. 4. Effect of PGE_2 and $PGF_{2\alpha}$ on isoproterenol-induced accumulation of cAMP in the myometrium. Endometrium was scraped from longitudinally incised uteri. Incubation was for 10 min in 1 mM theophylline. Values are means ±SEM of three determinations. Legends are the same as in Fig. 3.

stimulatory concentration of isoproterenol was used, suggesting a relation between the PGE_2 inhibitory site and the β-adrenergic receptor. These results are consistent with those of Marumo and Edelman (14) and of Shaw *et al.* (15), indicating that the inhibition of hormone effects produced by prostaglandins was associated with a decrease of tissue cAMP.

Since we were relating hormone action to known effects on myometrial contractivity, it was necessary to verify that the hormone-induced alterations of cAMP concentration were not occurring principally in the endometrium. As shown in Table 2, bovine endometrial strips failed to respond to isoproterenol but had a vigorous response to PGE_1 and PGE_2. Myometrial strips prepared from oophorectomized rats by scraping away the endometrium behaved exactly as did whole castrate uterus (Fig. 4). Isoproterenol induced cAMP accumulation, while both PGE_2 and $PGF_{2\alpha}$ were inhibitory. It was of interest that $PGF_{2\alpha}$ appeared to be a more potent inhibitor in the myometrial preparation.

The data best fit the hypothesis that prostaglandins E_2 and $F_{2\alpha}$ interfere with the β-adrenergic response in myometrium to inhibit cAMP formation and stimulate uterine contraction, while PGE_2 also stimulates other adenylate cyclases. In further support, it was found (Table 3) that oxytocin had no influence on PGE_2-induced accumulation of cAMP. When the inhibitory effect of propranolol was tested (Fig. 5), it was found that in concentrations sufficient to inhibit isoproterenol by 80%, in the presence of high concentrations of PGE_2 and isoproterenol, the concentration of cAMP remained high.

Such competitive studies cannot be conclusive because the prostaglandins may interact with the adenylate cyclase at a site proximal to the hormone-responsive site (14). However, the data of Figs. 3 and 4 also provide more direct evidence for distinct inhibitory and activating sites. If a single site were involved in both processes then, at the concentration required to inhibit uterine adenylate cyclase maximally, saturation should have occurred. If this were the case then adenylate cyclase stimulation should be similarly near

TABLE 3. *Effect of oxytocin on stimulation of cyclic AMP by prostaglandin E_2 in the uterus*

Treatment	cAMP (pmol/mg of protein)
Eagle's medium	7.9 ± 0.71
PGE_2 (50 μM)	27.7 ± 3.51
PGE_2 (50 μM) + oxytocin (10 mU/ml)	29.0 ± 3.31

Incubation was for 15 min in the presence of 5 mM theophylline. Values represent the mean of five determinations ±SEM.

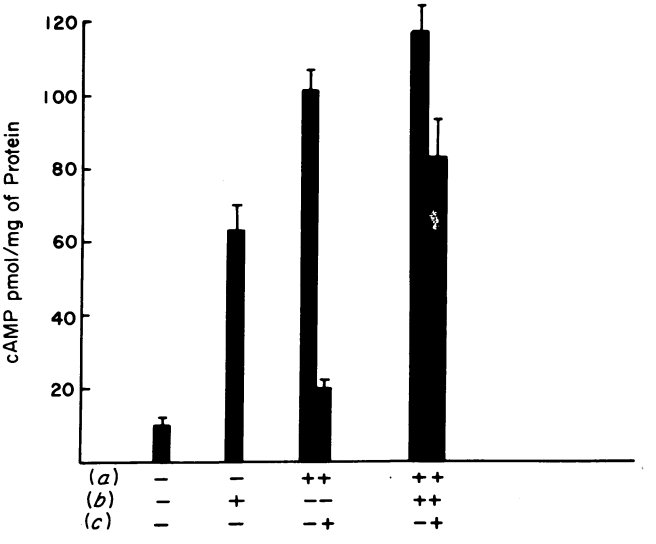

FIG. 5. Effect of propranolol on PGE_2-induced formation of cAMP. Incubation was for 10 min in 1 mM theophylline. Values are means ±SEM of four determinations. (*a*) 10 μM isoproterenol; (*b*) 50 μM PGE_2; (*c*) 5 μM propranolol.

maximal. However, much higher prostaglandin concentrations were necessary to stimulate cAMP maximally. A similar picture has emerged from studies of lipolysis regulated by hormones in epididymal fat pads where, in addition, a physical separation of the stimulatory and inhibitory adenylate cyclase sites was possible (16, 17).

While both endometrium and myometrium contain adenylate cyclases that can be stimulated by PGE, the β-adrenergic responsive cyclase is limited to the myometrium (Table 2, Fig. 4), indicating at least a partial physical separation.

The influences of catecholamines, oxytocin, and certain prostaglandins on myometrial contractility are well known. Studies with dibutyryl cAMP and phosphodiesterase inhibitors have demonstrated an inhibition of contractility (4). Recently, it has been reported that acetylcholine, a myometrial stimulator, significantly inhibited epinephrine-induced accumulation of cAMP (6). Those reports, in concert with the current data, have shown that the principal hormones affecting myometrial contractility interact in such a way as to alter myometrial cAMP concentration in a manner consistent with their physiological role. Added weight is therefore given to the theory that cAMP is the intracellular messanger that transduces the net hormonal influence in smooth muscle. Direct measurements in these laboratories have also shown that the increase in cAMP produced by isoproterenol results in activation of a myometrial protein kinase whose functions remain to be clarified.

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1. Butcher, R. W., Ho, R. J., Meng, H. C. & Sutherland, E. W. (1965) "Adenosine 3′:5′-monophosphate in biological materials. II. The measurement of adenosine 3′:5′-monophosphate in tissues and the role of the cyclic nucleotide in the lipolytic response of fat to epinephrine," *J. Biol. Chem.* **240**, 4515–4123.
2. Dobbs, J. W. & Robison, G. A. (1968) "Functional biochemistry of beta receptors in the uterus," *Fed. Proc.* **27**, 352.
3. Triner, L., Overweg, N. I. A., Hiatt, R. B. & Nahas, G. G. (1969) "The regulation of uterine contractions by catecholamines," *Physiologist* **12**, 377.
4. Mitznegg, P., Heim, F. & Meythaler, B. (1970) "Influence of endogenous and exogenous cyclic 3′:5′-AMP on contractile responses induced by oxytocin and calcium in isolated rat uterus," *Life Sci.* **9**, 121–128.
5. Triner, L., Vulliemoz, Y., Verosky, M. & Nahas, G. G. (1970) "The effect of catecholamines on adenyl cyclase activity in rat uterus," *Life Sci.* **9**, 707–712.
6. Triner, L., Vulliemoz, Y., Verosky, M. & Nahas, G. G. (1972) "Acetylcholine and the cyclic AMP system in smooth muscle," *Biochem. Biophys. Res. Commun.* **46**, 1866–1873.
7. Harbon, S. & Clauser, H. (1971) "Cyclic adenosine 3′:5′ monophosphate levels in rat myometrium under the influence of epinephrine, prostaglandins and oxytocin. Correlations with uterus motility," *Biochem. Biophys Res. Commun.* **44**, 1496–1503.
8. Karim, S. M. M., Hillier, K., Trussell, R. R., Patel, R. C. & Tamusange, J. (1970) "Induction of labour with prostaglandin E_2," *J. Obstet. Gynaecol. Brit. Commonw.* **77**, 200–210.
9. Sharma, S. K. & Talwar, G. P. (1970) "Action of cyclic adenosine 3′:5′-monophosphate *in vitro* on the uptake and incorporation of uridine into ribonucleic acid in ovariectomized rat uterus," *J. Biol. Chem.* **245**, 1513–1519.
10. Walton, G. M. & Garren, L. D. (1970) "An assay for adenosine 3′:5′-cyclic monophosphate based on the association of the nucleotide with a partially purified binding protein," *Biochemistry* **9**, 4223–4229.
11. Korenman, S. G. & Sanborn, B. M. (1971) in *Principles of Competitive Protein-Binding Assays*, eds. Odell, W. D. & Daughaday, W. H., (The J. B. Lippincott Co., Philadelphia, Pa.), pp. 89–107.
12. Krishna, G., Weiss, B. & Brody, B. (1968) "A simple sensitive method for the assay of adenyl cyclase," *J. Pharmacol. Exp. Ther.* **163**, 379–385.
13. Polacek, I. & Daniel, E. E. (1971) "Effect of α- and β-adrenergic stimulation on the uterine motility and adenosine 3′:5′-monophosphate level," *Can. J. Physiol. Pharmacol.* **49**, 988–998.
14. Marumo, F. & Edelman, I. S. (1971) "Effects of Ca^{++} and prostaglandin E_1 on vasopressin activation of renal adenyl cyclase," *J. Clin. Invest.* **50**, 1613–1620.
15. Shaw, J., Gibson, W., Jessup, S. & Ramwell, P. (1971) "The effect of PGE_1 on cyclic AMP and ion movements in turkey erythrocytes," *Ann. N.Y. Acad. Sci.* **180**, 241–260.
16. Butcher, R. W. & Baird, C. E. (1968) "Effects of prostaglandins on adenosine 3′:5′-monophosphate levels in fat and other tissues," *J. Biol. Chem.* **243**, 1713–1717.
17. Butcher, R. W. & Baird, C. E. (1967) "The relationship of prostaglandins and cyclic AMP levels," in *Prostaglandin Symposium of the Worcestor Foundation for Experimental Biology*, eds Ramwell, P. W. & Shaw, J. E. (Interscience, New York), pp. 42–48.