Effects of Prostaglandins PGE_2 and $PGF_{2\alpha}$ on the Adenyl Cyclase Activity of Various Segments of the Immature Rabbit Oviduct and Uterus (37360)

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Prostaglandins (PG) may play a physiological role in the transport of the ova and blastocysts through the oviduct and uterus. This role is not well-defined, nor is the mechanism by which prostaglandins act. One possibility is through affected sympathetic neuromuscular transmission. There are interesting studies demonstrating that PGE_1 and PGE_2 inhibited sympathetic neuromuscular transmission in several tissues in various species (1), that PGE_2 was released into the venous blood after stimulating the splenic nerve in dogs (2) and that PGE_1 inhibited the constriction of the rabbit oviduct after hypogastric nerve stimulation or administration of noradrenalin (3). A second possibility is that the effect is mediated through cAMP. It has been shown that adrenaline caused relaxation of the isolated rat uterus concomitant with an increase in cAMP and these effects could be blocked by propanolol (4). Isoproterenol increased rat uterus cAMP and this effect was blocked by the beta adrenergic blocking agent, propanolol (5–7) but not by the alpha adrenergic blocking agent, phentolamine (5). Oxytocin inhibited the increase in uterine cAMP produced by isoproterenol in ovariectomized rats (7). However, oxytocin itself, while causing the rat uterus to contract, did not alter cAMP levels and cAMP antagonized the contractions induced by this agent (8).

The relationship of the different prostaglandins to each other and to cAMP synthesis or action and to contraction of the oviduct and uterus is still not well defined. There is also much to be learned concerning the effects of drugs and of prostaglandins, in particular, on the various areas of the oviduct and uterus. Sandberg *et al.* (9–11) found that prostaglandins of the E and F series behaved differently in the human uterus and various segments of the Fallopian tube with respect to contraction and relaxation.

The present studies were initiated to determine the relative effects of PGE_2 and $PGF_{2\alpha}$ on adenyl cyclase activity of the oviductal ampulla and isthmus, the uterotubal junction and the uterus of the immature rabbit. It is well known that theophylline greatly increases cAMP concentrations by preventing its breakdown by phosphodiesterase, therefore, in order to measure only the effect of prostaglandins on synthesis, this agent also was used.

Materials and Methods. Tissues from immature female New Zealand rabbits weighing 1100–1300 g were used in these studies. The prostaglandins were obtained through the courtesy of Dr. Y. Takeda of Fuji Chemical Industries Ltd. of Takaoka, Toyama, Japan. The [8-^{3}H]adenine (17 Ci/mmole) was purchased from Schwarz-Mann of Orangeburg, NY.

Animals were killed by cervical dislocation and the uteri and oviducts were immediately removed and placed in petri dishes immersed in an ice bath. These tissues were then cleaned of all adhering tissues and divided into four anatomical areas, including the oviductal ampulla, oviductal isthmus, uterotubal junction and uterus. Each of these sections was then cut longitudinally to open the tube and transferred to vials containing 12.5 μCi [8-^{3}H]adenine (0.7×10^{-6} *M*) in 1 ml of modified Krebs–Ringer bicarbonate buffer (12) at pH 7.4. Preincubation was for 60 min, and incubation in the presence of the

prostaglandins, with or without theophylline, was for 15 min, according to the method of Fumagalli *et al.* (13). The ^{3}H-cAMP was isolated by column chromatography and precipitated with barium–zinc according to the technique of Krishna *et al.* (14). Radioactivity of ^{3}H-nucleosides and ^{3}H-cAMP was measured in a Packard TriCarb Liquid Scintillation Spectrometer Model 3320 using Bray's solution as modified in the method of Manara *et al.* (15). Counting efficiency was determined by internal standardization on randomly chosen samples and monitored on all of the samples, using automatic external standardization. The protein content of tissues was measured according to Lowry *et al.* (16), with crystalline bovine albumin as standard.

Results and Discussion. Prostaglandin PGE_2, in concentrations of 0.001–100 μg/ml, produced concentration-related increases in the conversion of labeled nucleotides to cAMP in the rabbit uterotubal junction (Fig. 1). The minimum effective concentration was between 0.001 and 0.01 μg/ml and maximum

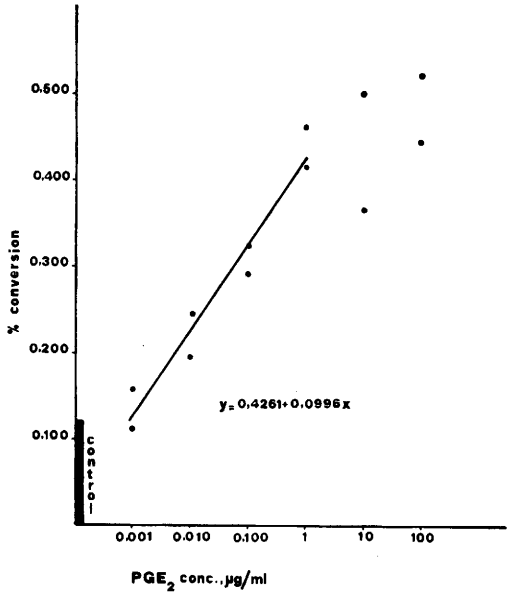

FIG. 1. Stimulation of cAMP synthesis in rabbit uterotubal junction by PGE_2. Statistical analysis and PGE_2 concentration–response regression line was calculated only for the four lower concentrations. The points represent individual observations.

TABLE I. Effect of PG_2 (10 μg/ml) and $PGF_{2\alpha}$ (10 μg/ml) on the Formation of ^{3}H-cyclic AMP in Three Different Segments of the Rabbit Oviduct.

Segments	Control		PGE_2			$PGF_{2\alpha}$		
	CPM/mg protein[a]	% Conversion[b]	CPM/mg protein	PG/C	% Conversion	PG/C	CPM/mg protein	PG/C	% Conversion	PG/C
Uterotubal junction	1702 ± 134[c] (6)	0.108 ± 0.012 (6)	4713 ± 421[d] (3)	2.8	0.340 ± 0.031[d] (3)	3.1	2312 ± 315 (3)	1.3	0.125 ± 0.007 (3)	1.1
Isthmus	1247 ± 191 (6)	0.149 ± 0.016 (6)	2775 ± 94[e] (3)	2.2	0.385 ± 0.008[d] (3)	2.6	1545 ± 98 (3)	1.2	0.153 ± 0.014 (3)	1.0
Ampulla	1335 ± 230 (6)	0.124 ± 0.009 (6)	1581 ± 197 (3)	1.2	0.218 ± 0.009[d] (3)	1.7	1740 ± 143 (3)	1.3	0.137 ± 0.014 (3)	1.1

[a] The results are expressed as counts in ^{3}H-cyclic AMP per mg protein.
[b] The results are expressed as percentage conversion of ^{3}H-nucleotides into ^{3}H-cyclic AMP in 15 min.
[c] Standard error of the mean; in parentheses are indicated the pool number: each experimental pool contained similar anatomical segments of oviducts of three rabbits.
[d] Significantly different ($p < 0.001$) from control.
[e] Significantly different ($p < 0.005$) from control.

stimulation was obtained with 1–10 μg/ml. Uterotubal junction tissue is more muscular than other areas of the oviduct and, therefore, could be expected to give stronger contractions and be stimulated to a greater extent than the ampulla or isthmus. To ensure maximal responses, prostaglandins at concentrations of 10 μg/ml were employed in subsequent studies.

Results shown in Table I demonstrate that the adenyl cyclase activities of the areas of the nontreated rabbit oviduct do not differ significantly from each other. Incubation with PGE_2, however, greatly stimulated cAMP synthesis in the uterotubal junction and isthmus, the increased CPM/mg protein were 2.8 and 2.2 times that of the controls in these tissues, respectively. Increases on the basis of percent conversion of the nucleotides to cAMP were 3.1 and 2.6 for the uterotubal junction and isthmus, respectively. The cAMP of the ampulla was not significantly altered on the basis of CPM/mg protein but it was significantly increased on the basis of percent conversion. These results demonstrate that various areas of the rabbit oviduct have differing capacities to respond to PGE_2 stimulation. PGF_{2a}, in the same concentration as that used for PGE_2, did not significantly alter the adenyl cyclase activity of any area of the oviduct. This indicates that even at a concentration that produced maximal stimulation with PGE_2, PGF_{2a} is inactive in this biological system.

Theophylline produced a threefold increase in adenyl cyclase activity in each of the three areas of the oviduct (Table II) when compared to control tissues (Table I). PGE_2 in the presence of theophylline significantly increased adenyl cyclase activity in all three segments of the oviduct (Table II). The increases in activity compared to control on the basis of percent conversion of nucleotides to cAMP or CPM/mg protein were approximately 2-, 3- and 4-fold for the ampulla, isthmus, and uterotubal junction, respectively. The addition of theophylline to the system increased the significance of the differences between the PGE_2 tissue pools and the controls. This experiment reemphasizes the difference in the magnitude of response to PGE_2 in the different areas of the oviduct and points to a gradient effect which is lowest at the ovarian end of the oviduct and reaches a maximum at the anatomical segment where ova and blastocysts accumulate and then are ejected into the uterine lumen.

The uterus responded to PGE_2 (10 μg/ml) by doubling the percent conversion of the labeled nucleotides into cAMP, but synthesis was unaffected by a similar concentration of PGF_{2a} (Table III). This stimulation by PGE_2 was less than that observed for the

TABLE II. Effect of Theophylline (T) (10^{-2} *M*) and Theophylline Plus PGE_2 (10 μg/ml) on the Formation of ^{3}H-cyclic AMP in Three Different Segments of the Rabbit Oviduct.

	Theophylline		Theophylline plus PGE_2			
Segments	CPM/mg protein[a]	% Conversion[b]	CPM/mg protein	T + PG/T	% Conversion	T + PG/T
Uterotubal junction	4236 ± 281[c] (3)	0.646 ± 0.084 (3)	18,582 ± 1139[d]	4.4	2.409 ± 0.288[e] (3)	3.7
Isthmus	4114 ± 266 (3)	0.907 ± 0.075 (3)	12,204 ± 3247[f] (3)	3.0	2.626 ± 0.427[f] (3)	2.9
Ampulla	3238 ± 408 (3)	0.754 ± 0.016 (3)	6905 ± 961[f] (3)	2.1	1.661 ± 0.180[g] (3)	2.2

[a,b,c] See footnotes of Table I.

[d] Signficantly different ($p < 0.001$) from control.

[e] Signficantly different ($p < 0.005$) from control.

[f] Signficantly different ($p < 0.05$) from control.

[g] Signficantly different ($p < 0.01$) from control.

TABLE III. Effect of PGE_2 (10 μg/ml) and $PGF_{2\alpha}$ (10 μg/ml) on Formation of ^{3}H-cyclic AMP in the Rabbit Uterus.[a]

Control	PGE_2	$PGF_{2\alpha}$
0.075 ± 0.002[b] (5)	0.155 ± 0.007[c] (3)	0.100 ± 0.014 (3)

[a] The results are expressed as percentage conversion of ^{3}H-nucleotides into ^{3}H-cyclic AMP in 15 min.

[b] Standard error of the mean; in parentheses are indicated the number of rabbits per group.

[c] Significantly different ($p < 0.01$) from control.

uterotubal junction.

Preliminary studies with isoproterenol incubated with immature rabbit uteri demonstrated that adenyl cyclase activity was increased in a manner similar to that reported for rat uteri (5–7). The effect of oxytocin on immature rabbit uteri was to decrease cAMP synthesis, which is in contrast to the report (8) that this smooth muscle contractor did not affect cAMP levels in the rat uterus. Expanded studies with these agents in the rabbit are in progress and the results will be submitted for publication in the near future.

Summary. Immature rabbit oviducts and uteri incubated in the presence of PGE_2 showed increases in the percent conversion of ^{3}H-nucleotides to ^{3}H-cAMP. The different segments of the oviduct had similar adenyl cyclase activities in the absence of prostaglandin. In the presence of PGE_2, however the degrees of response varied. The adenyl cyclase activities of oviductal ampulla, oviductal isthmus, uterotubal junction and uterus were 2, 3, 4, and 2 times those of their controls, respectively. Addition of theophylline to the incubation mixture enhanced the level of cAMP formation and the response to PGE_2·$PGF_{2\alpha}$, at a concentration (10 μg/ml) that produced a maximal response for PGE_2, did not significantly alter the adenyl cyclase activity in any area of the oviduct or uterus.

Isoproterenol increased and oxytocin decreased adenyl cyclase activity in the immature rabbit uterus.

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