THE STIMULATORY EFFECT OF PROSTAGLANDIN E_2 ON ADENYL CYCLASE IN THE BOVINE CORPUS LUTEUM

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1. Introduction

It has been shown that prostaglandin $F_2\alpha$, ($PGF_2\alpha$)* has a luteolytic effect when administered *in vivo* to rats [1] and guinea pigs [2]. These results suggest that this substance or one of the other prostaglandins might represent the uterine luteolytic factor which causes the periodic regression of the corpus luteum in several mammalian species. *In vitro* studies on the effects of $PGF_2\alpha$ and other prostaglandins on ovarian tissue, however, have not supported this idea [4, 5]. In fact, $PGF_2\alpha$ was found to increase rather than decrease steroidogenesis in whole rat ovaries incubated *in vitro* [4], and several prostaglandins: $PGF_2\alpha$; PGE_2; PGE_1; and PGA_1 increased steroidogenesis in incubating slices of bovine corpora lutea [5].

It is known that LH increases steroidogenesis in slices of bovine corpora lutea [6], and that this effect is mediated by cyclic AMP [7]. Recently it has been demonstrated that LH brings about the increase in cyclic AMP by activating the adenyl cyclase system [8].

The purpose of this work was to study the possibility that the stimulation of steroidogenesis by prostaglandins was also mediated by cyclic AMP. Specifically the effect of PGE_2 was assessed on the adenyl cyclase of homogenates of bovine corpora lutea. PGE_2 was chosen because it was the most potent of the prostaglandins in stimulating steroidogenesis in incubating slices of bovine corpora lutea [5].

* Abbreviations: $PGF_2\alpha$, prostaglandin $F_2\alpha$; PGE_2, prostaglandin E_2; PGE_1, prostaglandin E_1; PGA_1, prostaglandin A_1; LH, luteinizing hormone; cyclic AMP, adenosine 3′,5′-monophosphate.

2. Experimental procedure

2.1. *Materials*

Prostaglandin E_2 was a gift from the Upjohn Co., and bovine LH (NIH-LH-S-11) was supplied by the National Institutes of Health. α-^{32}P-ATP (specific activity 2–5 Ci/mmole) was purchased from the International Chemical and Nuclear Corp., and ^{3}H-cyclic AMP (specific activity 1–2 Ci/mmole) from Schwarz BioResearch. Other chemicals were reagent grade products. Corpora lutea were collected at slaughter from cows in the first 6 months of pregnancy (as determined by the size of the fetus [9]). A single corpus luteum was used in each experiment.

2.2. *Measurement of adenyl cyclase and progesterone synthesis*

The capsular tissue was removed from the corpus luteum and 1 g of luteal tissue was minced and then homogenized in 4 volumes of cold 0.02 M glycylglycine (pH 7.4) containing 0.01 M $MgSO_4$. The assay incubation procedure used was a modification of that of Sutherland et al. [10]. One tenth of a ml of homogenate suspension was added to 0.51 ml of a solution containing 25 μmoles of tris (pH 7.4), 24.4 μmoles theophylline, 1.2 μmoles ATP, 10 μCi α-^{32}P-ATP, 1.8 μmoles $MgSO_4$, 1.54 μmoles NaCl,

0.12 mg bovine serum albumin and the appropriate test substance. The reaction mixture was incubated with shaking, in air, at 30° for 5 min. The formation of cyclic AMP was assessed by measuring the conversion of α-^{32}P-ATP into ^{32}P-cyclic AMP. The ^{32}P-cyclic AMP was isolated by chromatography on Dowex 50 W-X8 and three precipitations with $BaSO_4$ according to the method of Krishna et al. [11]. The ^{32}P-cyclic AMP was counted at 80% efficiency in a scintillation counter, and the mass of carrier cyclic AMP recovered was measured by means of its maximal absorption at 257 nm. Protein concentration was determined by the method of Lowry et al. [12].

Slices of corpora lutea were incubated in the presence of acetate-1-^{14}C for two hours at 30° in Krebs-Ringer bicarbonate buffer, and the amount of progesterone synthesized was measured as previously described [7].

3. Results

3.1. *Purity of ^{32}P-cyclic AMP*

It has been claimed that the ^{32}P-cyclic AMP as determined by the Krishna method [11] can be significantly contaminated with other ^{32}P-labelled products [13]. We therefore undertook an assessment of the ^{32}P-labelled material isolated in experiments in which one aliquot of an homogenate was incubated with LH (10 μg/ml), a second with PGE_2 (10 μg/ml) and a third served as the control; the results are shown in table 1. The ^{32}P-cyclic AMP product, after the last precipitation of the Krishna method, was chromatographed on a cellulose thin-layer system in absolute ethanol: 0.1 M boric acid (3.5 : 1), adjusted to pH 4 with acetic acid [14]. The cyclic AMP zone was eluted with water and then incubated with phosphodiesterase prepared from bovine heart [15]. The products of this enzyme reaction were chromatographed in the system mentioned above. The specific activity of the ^{32}P-cyclic AMP was determined after the precipitation step and compared to the specific activity of the ^{32}P-cyclic AMP after chromatography and the specific activity of the ^{32}P-5′-AMP formed by the action of phosphodiesterase on ^{32}P-cyclic AMP.

The specific activity of the ^{32}P-cyclic AMP after chromatography and the ^{32}P-5′-AMP were essentially the same as the original ^{32}P-cyclic AMP for the control, LH or PGE_2 treated samples indicating that the radioactivity determined by the Krishna method was radiochemically pure ^{32}P-cyclic AMP.

3.2. *Effect of PGE_2 on adenyl cyclase activity and steroidogenesis*

Aliquots of an homogenate equivalent to 20 mg of luteal tissue were incubated under control conditions, with PGE_2 (10 μg/ml), or with LH (10 μg/ml); the results are shown in table 2. In every experiment PGE_2 and LH each increased the amount of cyclic AMP formed and these increases were statistically significant.

In three experiments, tissue slices and a homogenate were prepared from the same corpus luteum to confirm the stimulatory effects of PGE_2 on steroidogenesis [5] and to compare this stimulation with the extent of the activation of adenyl cyclase. The results shown in table 2 confirmed that PGE_2

Table 1.
Test of the radiochemical purity of ^{32}P-cyclic AMP formed in the assay of adenyl cyclase.

Step	^{32}P-Cyclic AMP specific activity (dpm/mg)		
	Control	LH	PGE_2
1. After the procedure of Krishna [11]	5820	11,700	12,000
2. After thin-layer chromatography	5070	12,100	9,490
3. After conversion of the ^{32}P-cyclic AMP into ^{32}P-5′-AMP with phosphodiesterase	5320	10,800	10,200

The table illustrates the specific activity of the ^{32}P-cyclic AMP isolated by the method of Krishna [11] and the changes that take place when this material is chromatographed, and then converted into ^{32}P-5′-AMP by phosphodiesterase and chromatographed again.

Table 2
Effect of PGE_2 on adenyl cyclase and progesterone synthesis in bovine corpora lutea.

Exp.	Adenyl cyclase activity (pmoles cyclic AMP/mg protein)			Progesterone synthesis (μg/g tissue)			Progesterone synthesis (dpm/g tissue) $\times 10^{-3}$		
	Control	PGE_2	LH	Control	PGE_2	LH	Control	PGE_2	LH
1	15.6	31.1	64.8	37.1	180	221	58.2	600	1070
2	11.8	32.1	31.5	90.9	122	123	46.1	99.8	211
3	27.8	42.8	78.0	127	285	458	155	286	487
4	13.5	24.1	23.4						
5	12.1	27.8	60.8						
6	12.1	27.2	18.7						
7	15.4	44.5	23.1						
8	19.9	32.9	31.2						
9	13.2	23.9	47.2						
10	30.4	62.8	75.0						
Mean ± S.E.*	17.2 ± 2.0	34.9 ± 3.6	45.4 ± 6.8						
p**		<0.0001	<0.001						

* Mean ± standard error of the mean.
** p was calculated using a t test of the mean difference at 9 degrees of freedom.

stimulates both the mass of progesterone synthesized and the incorporation of ^{14}C from acetate-1-^{14}C into the steroid. They also showed that the extent of stimulation of steroidogenesis correlates well with the increase in cyclic AMP formed in the same tissue.

This increase in the accumulation of cyclic AMP produced by PGE_2 could theoretically be due to either an activation of the adenyl cyclase system or an inhibition of the phosphodiesterase enzyme system. The inclusion of a high concentration of theophylline (0.04 M) in the assay incubation, and the short incubation time of 5 min were chosen to block the phosphodiesterase activity of homogenates of corpora lutea [8]. To assess the effectiveness of this block, exogenous, ^3H-labelled cyclic AMP was included in the reaction mixture and the extent of its destruction was measured simultaneously with the production of ^{32}P-cyclic AMP. The adenyl cyclase and phosphodiesterase activity of 1, 2 or 4 mg of homogenate protein from three experiments are shown in fig. 1. There was essentially no destruction of the ^3H-cyclic AMP and the adenyl cyclase activity was proportional to the amount of enzyme protein. The increased accumulation of ^{32}P-cyclic AMP brought about by PGE_2 under these conditions indicates that this

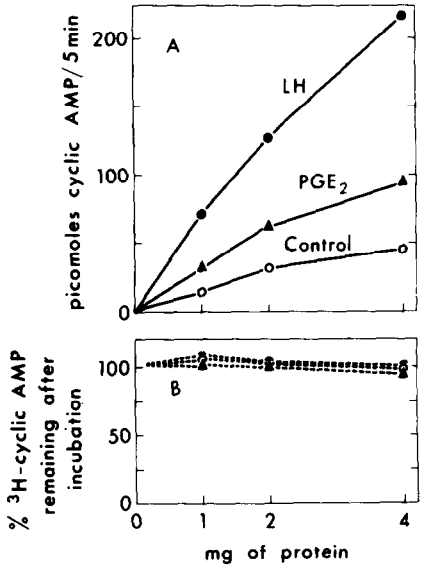

Fig. 1. A. Adenyl cyclase activity as a function of the mg of homogenate protein used. The symbol ○ refers to control assays. ▲ refers to assays with PGE_2; and ● refers to assays with LH. The points represent the means of two determinations.
B. The destruction of ^3H-cyclic AMP in adenyl cyclase shown in A. The symbols are the same as in A. The points represent the means of two determinations.

substance acts by activating the adenyl cyclase rather than by inhibiting the phosphodiesterase system.

4. Discussion

The results of this study suggest that PGE_2 stimullates steroidogenesis via the mediation of cyclic AMP. They also demonstrate that this prostaglandin brings about an increase in cyclic AMP by activating the adenyl cyclase rather than by inhibiting the phosphodiesterase system. Prostaglandins have been shown previously to effect the accumulation of cyclic AMP in incubations of slices and homogenates of other tissues [16, 17], but it has never been clearly shown whether these effects were due to an influence on the adenyl cyclase or the phosphodiesterase.

The functional significance of this effect of PGE_2 in corpora lutea is unknown. It could be a mediator of the action of LH, acting between LH and its effect on adenyl cyclase. If this were true, it would require that LH increase the concentration of PGE_2, or other prostaglandins in the luteal tissue. Prostaglandin-like material has been reported many years ago to be present in cow and sheep ovaries [18] indicating that such a functional relationship might exist.

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