Adrenocortical Steroidogenesis: the Effects of Prostaglandins

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ABSTRACT Prostaglandins E_1 and E_2 significantly stimulated the synthesis of aldosterone, corticosterone, and to a lesser degree, cortisol in the outer slices of beef adrenal tissue. PGA, $PGF_{1\alpha}$, and $PGF_{2\alpha}$ were ineffective.

PGE_1 was found to stimulate steroidogenesis in a manner similar to that of adrenocorticotropin (ACTH) in (*a*) needing calcium, (*b*) being inhibited by puromycin but not actinomycin D, (*c*) increasing the levels of cyclic AMP, and (*d*) not having an additive effect to exogenous cyclic AMP. PGE_1 did not produce an additive effect with either submaximal or maximal amounts of ACTH but did have an additive effect with angiotensin.

These results are in keeping with the hypothesis that PGE_1 shares a receptor site on the plasma membrane with ACTH.

INTRODUCTION

Prostaglandins have been found to stimulate hormonal release from various glands, including the anterior pituitary (1), the thyroid (2), the pancreas (3), and the ovary (4). Conflicting data have been published concerning their effects upon the adrenal cortex. An increase in steroidogenesis was noted both in vitro (5) and in vivo (6) in the rat but the latter study failed to show a response in the hypophysectomized animal, suggesting that prostaglandins act by stimulating adrenocorticotropin (ACTH) release and not directly upon the adrenal. Since the rat adrenal differs considerably in its response from the adrenal of most higher species (7), a study more relevant to human physiology may be one that involved sheep wherein PGE infusions into autotransplanted adrenals produced an over-all decrease in aldosterone secretion, though considerable variation was observed (8). Moreover, that study was done when the animals were sodium deficient, a situation wherein the responses to various stimuli may be altered (9).

We have examined the effects of prostaglandins upon steroids synthesis in beef adrenal slices, an in vitro system which we have found to respond to various stimuli —ACTH, angiotensin, potassium, and sodium—in a manner qualitatively similar to the responses of human adrenal tissue in vivo (10). These studies demonstrate a definite action of certain prostaglandins and suggest a possible role for these hormones in the control of steroidogenesis.

METHODS

Tissue preparation and incubation. As detailed in the previous paper (11), for each study the outer two slices of 15–20 fresh beef adrenal glands were combined, minced, and divided into 500-mg portions for incubation. 24–40 separate portions were incubated with at least 4 portions for each point in the experiment. After a 1 hr preincubation, the Krebs-Ringer bicarbonate medium was replaced, the various stimulatory agents being compared were added, and the tissues incubated for 2 hr.

Stimulatory agents. Prostaglandins E_1, E_2, A_1, $F_{1\alpha}$, and $F_{2\alpha}$ were provided by Dr. James Weeks, The Upjohn Company (Kalamazoo, Mich.) and dissolved in 0.1% ethanol. Porcine ACTH and synthetic angiotensin II were the same as used in the previous experiments. Doses were at least 10-fold greater than the lowest doses previously noted to produce significant effects (10), to ensure that suppression by other agents, e.g., puromycin, would be demonstrable. Cyclic AMP was dissolved in Krebs-Ringer bicarbonate medium and brought to pH 7.4 by addition of 1 N sodium hydroxide.

Assay procedures. Portions of the media were analyzed for steroids by a double-isotope derivative assay (12), slightly modified (11). Cyclic AMP was measured by a radioimmunoassay (13). Values are expressed as micrograms of steroid or picomoles of cyclic AMP in the incubation medium/gram of tissue per total time of incubation. The mean and standard deviation of the steroid values in each group of vessels were compared with those of the

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control specimens simultaneously incubated. Statistical evaluation was by Dunnett's test for multiple comparisons with a control (14).

RESULTS

These studies were designed first to demonstrate an effect of prostaglandins upon steroid synthesis and second to examine the possible mechanism of action of these agents, in comparison to what is known about ACTH and angiotensin.

The effect of various prostaglandins. In Fig. 1, the effects of 100 μg of five prostaglandins upon the synthesis of aldosterone, corticosterone, and cortisol are shown in comparison to the effects of angiotensin, 20 μg and ACTH, 2 U/g tissue. In another experiment comparing the five prostaglandins, PGE_2 had a slightly greater effect than did PGE_1, PGA had an insignificant effect, $PGF_{1\alpha}$ decreased aldosterone synthesis by 15% and corticosterone synthesis by 36%, and $PGF_{2\alpha}$ was without effect. In view of its inhibitory effect, $PGF_{1\alpha}$, 50 μg, was combined with ACTH, 2 U along with separate incubations of each agent. Although in this experiment $PGF_{1\alpha}$ alone decreased aldosterone synthesis by 22%, it had no effect upon the degree of stimulation of aldosterone, corticosterone, or cortisol synthesis produced by ACTH.

The effect of varying amounts of PGE_1. In the remainder of the studies, only PGE_1 was used. The results of three experiments with varying amounts of PGE_1 are shown in Fig. 2. No effect was noted with 0.01 μg/g tissue and an insignificant effect with 0.1 μg/g tissue. However amounts of 1.0 μg or more always increased steroidogenesis.

PGE_1 stimulated the synthesis of aldosterone, corticosterone, and cortisol in each experiment. However the effect upon aldosterone was almost always greater than upon the other two. In 13 different experiments with

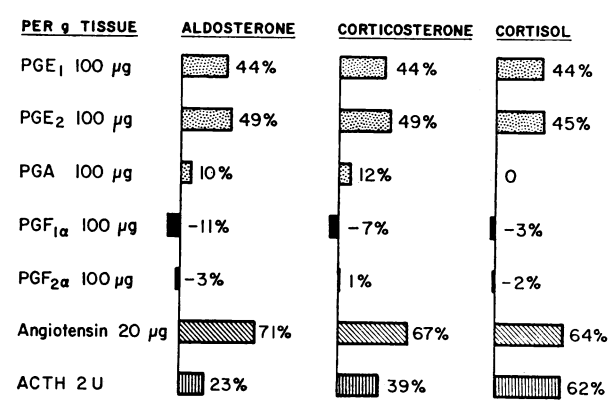

FIGURE 1 The effects of five prostaglandins, angiotensin, and ACTH upon steroid synthesis in beef adrenal tissue. The percentages are the change in synthesis from control vessels.

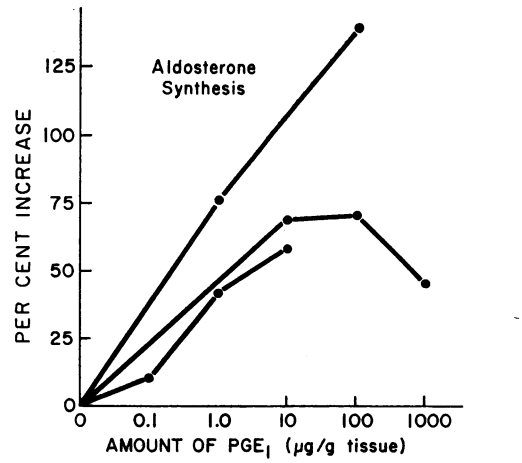

FIGURE 2 The effect of increasing amounts of PGE_1 upon aldosterone synthesis in three separate experiments.

amounts of PGE_1 varying from 1 to 100 μg/g tissue, the mean increases in synthesis above control were: aldosterone 63%, corticosterone 37%, and cortisol 38%. In three experiments with 1.0 μg/g tissue, aldosterone increased an average of 59% whereas cortisol increased 18%. On a molar basis, PGE_1 was about equal to angiotensin II in its effect upon aldosterone synthesis in this preparation (10).

Having demonstrated an effect of PGE_1 upon steroidogenesis, four approaches were taken to examine its mode of action: (*a*) combining it with other stimuli: ACTH, cyclic AMP, angiotensin, or increased concentrations of potassium, (*b*) examining the effect of inhibitors of protein and RNA synthesis, (*c*) determining the need for calcium, and (*d*) measuring cyclic AMP levels.

Combinations of PGE_1 and other stimuli

ACTH or cyclic AMP. As shown in Table I, PGE_1 1 μg/g tissue, did not produce an additive effect to that of a submaximal dose of ACTH in experiment 1 nor did PGE_1, 50 μg/g tissue stimulate steroidogenesis beyond that noted with a presumably maximal dose of ACTH in experiment 2. Similarly, in experiment 3, PGE_1 did not potentiate the effect of exogenous cyclic AMP, 1 mM/g tissue.

Angiotensin. ACTH and cyclic AMP are thought to affect adrenal steroidogenesis in a similar manner, whereas angiotensin appears to act differently (11). Therefore PGE_1 was next combined with submaximal doses of angiotensin II (Table II). The results of experiments 4 and 5, similar to those of four other studies, indicate that PGE_1 produces an additive effect to that of angiotensin II. The levels of all three steroids achieved with the combination of PGE_1 plus angiotensin are significantly greater than those achieved with each agent

TABLE I

The Effect of PGE_1 in Combination with ACTH or Cyclic AMP

Experiment	Stimulant Name	Stimulant Dose	Aldosterone Mean	Aldosterone SD	Corticosterone Mean	Corticosterone SD	Cortisol Mean	Cortisol SD
		per g tissue			*µg/g tissue per 2 hr incubation*			
1	None		0.91	0.12	14.42	1.09	1.56	0.12
	PGE_1	1 µg	*1.18*	*0.07**	16.77	0.89	*2.42*	*0.24*
	ACTH	2 U	*1.11*	*0.09*	15.79	1.20	*2.47*	*0.22*
	PGE_1	1 µg and	*1.14*	*0.05*	15.28	1.15	*2.36*	*0.26*
	ACTH	2 U						
	ACTH	4 U	*1.40*	*0.12*	*17.40*	*2.32*	*2.95*	*0.27*
2	None		0.96	0.08	10.49	1.09	1.68	0.13
	PGE_1	50 µg	*1.78*	*0.24*	11.45	0.79	2.05	0.18
	ACTH	20 U	*2.01*	*0.15*	*14.39*	*0.70*	*4.35*	*0.77*
	ACTH	40 U	*2.07*	*0.18*	*14.06*	*0.88*	*4.01*	*0.68*
	PGE_1	50 µg and	*2.02*	*0.27*	*15.29*	*0.72*	*4.26*	*0.59*
	ACTH	20 U						
3	None		1.56	0.15	5.49	0.58	1.58	0.16
	PGE_1	1 µg	*2.47*	*0.25*	*7.28*	*0.59*	1.85	0.07
	Cyclic AMP	1 mM	*1.98*	*0.10*	*7.59*	*1.47*	*1.96*	*0.24*
	PGE_1	1 µg and	*2.58*	*0.17*	*8.33*	*0.72*	*2.06*	*0.10*
	Cyclic AMP	1 mM						

* Results in italics differ statistically from the control level with P value less than 0.05.

individually. These differences between ACTH and angiotensin with PGE_1 are shown in Fig. 3 for submaximal doses and in Fig. 4 for maximal doses. The doses of ACTH and angiotensin depicted in Fig. 4 are considered to be maximal because no greater effect was noted with doses twice as large.

Potassium. As shown in Table III, PGE_1, 50 µg/g tissue, did not produce an additive effect on aldosterone synthesis to that of an increased potassium concentration of 7.6 mEq/liter. In this experiment aldosterone synthesis could be further stimulated by an even higher potassium concentration.

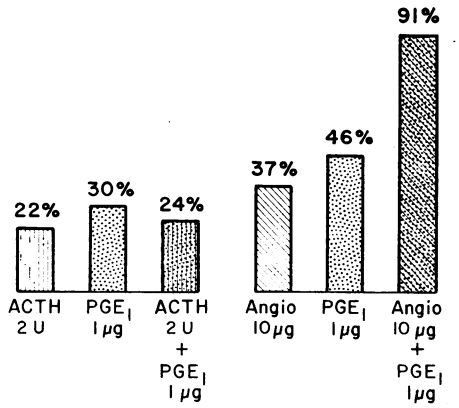

FIGURE 3 The effect of submaximal doses, individually and together, of ACTH and PGE_1, on the left, and of angiotensin II and PGE_1, on the right. Each set of three bars is a separate experiment.

Inhibitors of protein and RNA synthesis

Puromycin. In a manner similar to that observed with ACTH, angiotensin and increased potassium concentration (11), puromycin 10^{-6} M completely inhibited the stimulation of steroid synthesis by PGE_1. The effect upon aldosterone synthesis in one experiment is shown in Fig. 5 and a similar inhibition of the stimulation of aldosterone, corticosterone, and cortisol synthesis was observed in two other studies.

TABLE II

The Effect of PGE_1 in Combination with Angiotensin II

Experiment	Stimulant Name	Stimulant Dose	Aldosterone Mean	Aldosterone SD	Corticosterone Mean	Corticosterone SD	Cortisol Mean	Cortisol SD
		per g tissue			*µg/g tissue per 2 hr incubation*			
4	None		2.19	0.23	6.06	1.29	2.26	0.23
	PGE_1	1 µg	*3.20*	*0.19**	*9.52*	*2.29*	3.24	0.22
	Angiotensin	10 µg	*3.01*	*0.21*	8.51	1.15	3.25	0.27
	PGE_1	1 µg and	*4.18*	*0.26*	*14.20*	*3.14*	*6.02*	*0.80*
	Angiotensin	10 µg						
	Angiotensin	100 µg	*4.43*	*0.52*	*16.82*	*2.30*	*6.23*	*1.25*
5	None		0.91	0.12	14.42	1.09	1.56	0.12
	PGE_1	50 µg	*1.18*	*0.07*	*16.77*	*0.89*	*2.42*	*0.24*
	Angiotensin	1000 µg	*1.41*	*0.10*	*18.72*	*1.67*	*2.68*	*0.18*
	Angiotensin	2000 µg	*1.38*	*0.05*	*17.46*	*0.88*	*2.38*	*0.24*
	PGE_1	50 µg and	*1.79*	*0.12*	*20.15*	*1.80*	*3.62*	*0.31*
	Angiotensin	1000 µg						

* Results in italics differ statistically from the control level with P value less than 0.05.

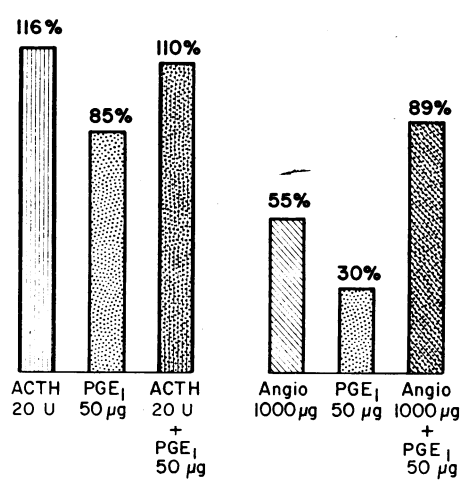

FIGURE 4 The effect of maximal doses, individually and together, of ACTH and PGE_1, on the left, and of angiotensin II and PGE_1, on the right. Each set of three bars is a separate experiment.

TABLE III

The Effect of PGE_1 in Combination with an Increased Potassium Concentration

PGE_1 dose	Potassium concentration	Aldosterone		Corticosterone		Cortisol	
		Mean	SD	Mean	SD	Mean	SD
μg/g tissue	*mEq/liter*			*μg/g tissue per 2 hr incubation*			
None	5.2	1.59	0.18	8.55	0.42	2.11	0.18
50 μg	5.2	*2.39*	*0.20**	*14.65*	*1.12*	*2.88*	*0.16*
None	7.6	*2.64*	*0.35*	*14.44*	*1.30*	2.05	0.25
50 μg	7.6	*2.62*	*0.34*	*15.12*	*1.16*	2.78	0.29
None	9.8	*3.48*	*0.29*	*19.44*	*2.00*	2.16	0.22

* Results in italics differ statistically from the control level with P value less than 0.05.

Actinomycin D. In three experiments, actinomycin D, 5×10^{-4} M, had little if any affect upon the steroidogenic effect of PGE_1. In one study, shown in Table IV, blunting of the stimulation of aldosterone synthesis but not of corticosterone or cortisol was observed with actinomycin D. In the other two studies, not even this partial inhibition was observed.

The need for calcium

The stimulation of steroidogenesis by PGE_1 was prevented in the absence of calcium from the incubation medium.

The effect of PGE_1 upon cyclic AMP

As shown in Table V, the levels of cyclic AMP in the incubation media increased in association with the stimulation of steroidogenesis by PGE_1. In these and two other experiments, one at each interval of incubation, the effects of PGE_1 were quite similar to those of ACTH.

DISCUSSION

These studies clearly show a stimulation of beef adrenal steroidogenesis in vitro by prostaglandins E_1 and E_2.

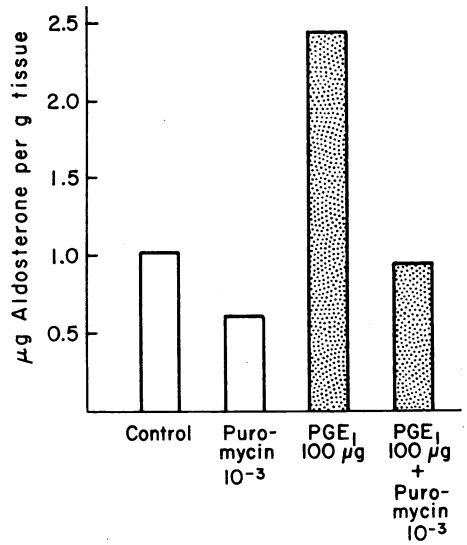

FIGURE 5 The effect of puromycin upon the stimulation of aldosterone synthesis in beef adrenal tissue by PGE_1.

Whether these findings have any relevance to human physiology is unknown since there are no published data on the effects of the prostaglandins upon adrenal steroids in man though prostaglandins have been found within human adrenal tissue (15). The results of similarly performed experiments with this in vitro model with ACTH, angiotensin and potassium have, in general, been compatible with what is known about the control of steroidogenesis in man (10).

The effect of PGE_1 upon adrenal steroidogenesis in this in vitro model requires doses of 1 μg or more/g of tissue. Whether such doses are "physiologic" is conjectural since plasma and tissue levels have not been measurable with accuracy. The availability of a radioimmunoassay (16) may make it possible to determine "physiologic" levels. It should be noted that microgram quantities of PGE_1 were needed for a steroidogenic effect in both in vitro (5) and in vivo (6) studies on the

TABLE IV

The Effects of Actinomycin D upon the Action of PGE_1

PGE_1 dose	Agent	Aldosterone		Corticosterone		Cortisol	
		Mean	SD	Mean	SD	Mean	SD
				μg/g tissue per 2 hr incubation			
—	—	2.08	0.20	10.24	1.05	1.89	0.17
—	Actinomycin D 5×10^{-4} M	1.90	0.15	11.74	1.29	2.05	0.48
50 μg	—	*4.28*	*0.35**	*19.73*	*1.75*	*2.81*	*0.18*
50 μg	Actinomycin D 5×10^{-4} M	*3.17*	*0.35*	*18.76*	*1.05*	*2.67*	*0.28*

* Results in italics differ statistically from the control level with P value less than 0.05.

TABLE V

The Effect of PGE_1 upon Aldosterone and Cyclic AMP Levels in Media of Beef Adrenal Slice Incubations

Stimulant	Aldosterone		Corticosterone		Cortisol		Cyclic AMP	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
			μg/g tissue per incubation				*pmoles/g tissue per incubation*	
2 hr incubation								
Control	2.08	0.20	10.24	1.05	1.89	0.17	57.5	5.2
PGE_1, 50 μg	*4.28*	*0.75**	*19.55*	*1.46*	*3.56*	*0.28*	*83.5*	*6.4*
ACTH, 2 U	*3.21*	*0.18*	*17.38*	*1.60*	*4.08*	*0.57*	*105.6*	*12.5*
15 min incubation								
Control	1.02	0.08	4.44	0.40	1.10	0.06	81.2	5.9
PGE_1, 50 μg	*1.65*	*0.15*	*6.10*	*0.52*	*1.85*	*0.20*	*215.5*	*12.5*
ACTH, 2 U	*1.76*	*0.13*	*6.36*	*0.26*	*2.23*	*0.11*	*233.0*	*20.2*

* Results in italics differ statistically from the control level with P value less than 0.05.

rat. Similar amounts of prostaglandins were needed to demonstrate stimulation of other endocrine tissue in vitro, including the anterior pituitary (1), the thyroid (2), and the ovary (4). Although larger amounts of stimulatory agents are usually required in vitro, similar doses of prostaglandins were needed to demonstrate their effects in vivo, including the stimulation of growth hormone secretion in man (17).

In the studies reported here, PGE_1 and E_2 were most active in stimulating steroidogenesis, whereas $PGF_{1\alpha}$ and $PGF_{2\alpha}$ were without effect. Similar differences have been observed in studies on the thyroid (18) and anterior pituitary (1), although $PGF_{2\alpha}$ was effective upon the rat adrenal gland (5). More must be known about the nature and distribution of the various prostaglandins before the meaning of these differences is understood.

The studies on the possible mechanism of action of PGE_1 suggest that it may stimulate adrenal steroidogenesis in a manner similar to that proposed for ACTH which is thought to involve the following sequence: (*a*) binding to the plasma membrane, (*b*) activation of adenyl cyclase, (*c*) production of cyclic AMP, (*d*) release of a protein phosphokinase, and (*e*) synthesis of a protein which regulates the conversion of cholesterol to pregnenolone within the mitochondria (19).

The similarities between the action of PGE_1 and ACTH noted in this study are: (*a*) PGE_1 does not produce an additive effect to either submaximal or maximal amounts of ACTH or cyclic AMP, supporting a common path or site of action. On the other hand, PGE_1 does have an additive effect with angiotensin II which appears to act in a different manner (11); (*b*) the action of PGE_1 requires the presence of calcium; (*c*) an inhibitor of protein synthesis, puromycin, blocks the effect of PGE_1 but an inhibitor of DNA synthesis, actinomycin D, does not; (*d*) levels of cyclic AMP are increased when PGE_1 acts upon adrenal tissue.

Prostaglandins have been found to affect cyclic AMP in a variety of tissues (20). Early studies, primarily with fat cells, gave rise to an hypothesis that prostaglandins were released within tissues by the action of cyclic AMP, increased after hormonal stimulation (21). The prostaglandins were postulated to then exert an inhibitory action upon adenyl cyclase, thereby limiting the action of the hormone via an "internal" negative feedback.

This hypothesis was constructed to explain the apparent paradox of the action of prostaglandin in several tissues: in fat cells, PGE_1 inhibits the effect of various lipolytic agents (20) whereas in smaller doses PGE_1 itself induces lipolysis (22); in the isolated toad bladder and rabbit renal tubule, PGE_1 inhibits the action of vasopressin upon increasing water permeability, whereas PGE_1 itself increases water permeability (23).

However a more attractive hypothesis for the action of prostaglandins upon endocrine tissue has been suggested wherein they involve the activation of adenyl cyclase in a common pathway with the appropriate trophic hormone (23, 24). The results of the present study further suggest a sharing of the receptor site on the plasma membrane between PGE_1 and one or more other stimulatory agents. Thereby, PGE_1 could stimulate activity within the cell by activating adenyl cyclase but at the same time competitively inhibit the binding or other necessary steps in the action of other trophic agents which also act via the adenyl cyclase mechanisms. Within the adrenal cortex, this would be at the receptor site for ACTH. Within the thyroid, there appears to be competition between thyroid-stimulating hormone (TSH) and PGE_1 for a common adenyl cyclase receptor site (25).

This theory would appear to explain the paradox wherein prostaglandins stimulate adenyl cyclase in almost every tissue examined but at the same time appear to inhibit the action of other trophic agents.

There are many prostaglandins some of which act differently in the same tissue. The theory supported by this study may not apply to all tissues, to all prostaglandins or to all other trophic agents. Prostaglandins may be involved in the control of steroidogenesis in other ways than the direct stimulation shown in this in vitro study. Thus ACTH may stimulate the formation or release of prostaglandins from adrenal tissue (26). Further, PGE_1 causes a release of renin which, in turn by activating angiotensin, may stimulate steroidogenesis (27).

PGE_1 appears to act primarily upon aldosterone synthesis in these in vitro studies using the outer two slices of beef adrenal cortex. This preparation contains all of the aldosterone producing tissue and only part of the cortisol producing tissue so that caution should be taken in interpretation of these data in support of a selective role of PGE_1. More pertinent studies upon steroid secretion in vivo in both man and other higher species will hopefully define the exact role of prostaglandins in steroidogenesis.

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