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THE EFFECT OF PROSTAGLANDINS ON 11-β HYDROXYLASE ACTIVITY IN BOVINE ADRENOCORTICAL MITOCHONDRIA[1]

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Summary: The effects of prostaglandins of the E, F, and A series on 11-β hydroxylase activity was examined by monitoring the conversion of 11-deoxycortisol to cortisol. PGA_1 and PGA_2 were found to significantly increase steroid 11-β hydroxylation at the three doses tested. In general, prostaglandins of the E and F series inhibit cortisol formation when compared with untreated controls. These findings suggest that prostaglandin-induced alterations in adrenal steroidogenesis are in part the result of a direct interaction between prostaglandins and enzymes of the steroid biosynthetic pathway.

Two lines of evidence suggest that prostaglandins (PGs) are physiological mediators of adrenal steroidogenesis. First, the *in vitro* incubation of exogenous PGs or prostacyclin with adrenocortical tissue results in enhanced steroid biosynthesis in several mammalian species including humans. (1-4) Second, adrenocorticotropin (ACTH) induces *de novo* PG biosynthesis in isolated adrenocortical cells (5,6). The possible physiological relevance is obvious but questionable since a mechanism for PG action has not been clearly identified. Characterization of such a mechanism and the site or sites of action is prerequisite to assigning a physiological role to prostaglandins in the adrenal cortex.

One possible mechanism that has been investigated involves the direct interaction of PGs with enzymes of the biosynthetic pathway. In this regard, Rolland and Chambaz (7) have demonstrated a two-fold increase in cholesterol side chain cleavage (CSCC) activity in bovine adrenocortical mitochondria

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treated with various PGs in the presence of calcium. These results, however, were contradicted by Mason et al. (8) using rat adrenal mitochondria. They found no change in CSSC activity attributable to the influence of prostaglandins and recently, PGs were demonstrated ineffective in altering 21-hydroxylation (9).

We examine the effect of PGs on another reaction in the steroid biosynthetic pathway, 11-β hydroxylation, by quantitating the conversion of 11-deoxycortisol to cortisol, the major glucocorticoid synthesized by this species. We demonstrate that prostaglandins alter 11-β hydroxylase activity in bovine adrenocortical mitochondria and that the nature and extent of these alterations depends on the specific prostaglandin tested.

MATERIALS AND METHODS

Adrenals were collected at a local slaughterhouse within one hour of the animal's death, immediately defatted and placed in ice cold 0.32M sucrose. Mitochondria were isolated by a modification of the procedure of Cammer et al. (10). Briefly, each gland was bisected longitudinally, demedullated and the fasciculata - reticularis layers scraped from the glomerulosa layer. All operations were performed at 0-5°C. Cortical tissue was suspended in a homogenization media consisting of 0.32M sucrose, 2mM MOPS buffer and 1mM EGTA (pH 7.2). Tissue scrapings were homogenized in a Potter-Elevjhem homogenizer. The homogenate was centrifuged (900 x g; 4°C; 10 min.) in a Beckman J2-21 preparative centrifuge. The 900 x g pellet was discarded and the supernatant recentrifuged (9000 x g; 4°C; 10 min.) to pellet the mitochondrial fraction. The mitochondrial pellet was then resuspended in homogenization buffer and recentrifuged (9000 x g; 4°C 10 min.). All mitochondria were used within 3 hrs of final preparation.

Mitochondrial samples were dissolved in 10% deoxycholate and the protein concentration determined by the biuret method. Non-specific absorbance was corrected for by using the cyanide treatment described by Szarkowska and Klingenberg (12).

Incubations were terminated after 10 minutes by addition of 3.0 ml of dichloromethane. Termination in this manner allowed simultaneous extraction of cortisol. Extraction efficiency was determined using ^{3}H-cortisol (New England Nuclear) and consistently found to be > 89%. A 100 µl aliquot of each extract was removed, dried under air and assayed for cortisol content by radioimmunoassay using a second antibody precipitation technique. The anti-cortisol antiserum (Bio Ria) exhibits a 6% cross-reactivity with 11-deoxycortisol. Data was analyzed by use of student t test.

RESULTS

Figure 1 depicts typical oxygraph traces obtained for bovine adrenocortical mitochondria isolated according to the procedure described in Materials and Methods. With our isolation procedure similar ADP:O ratios were

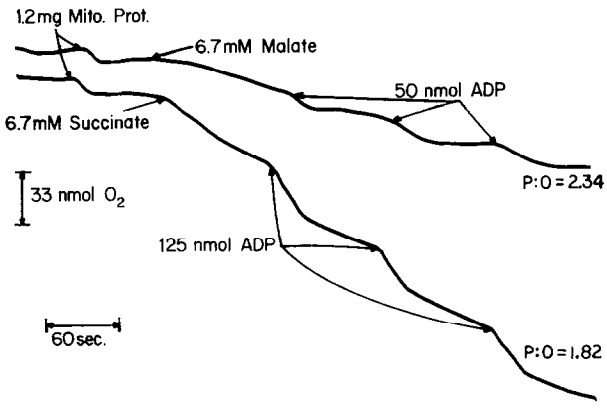

Figure 1. Oxygen consumption was monitored polarographically using a Clark electrode. Tracings were recorded on a Gilson Model K-IC oxygraph. The media in which these studies were undertaken consisted of 0.32M, 2mM MOPS, 20mM KCl, 10mM KH_2PO_4, 5mM $MgCl_2$, and 1mM EGTA (pH 7.2). ADP:O ratios were determined using the method of Estabrook (11).

routinely obtained and emphasis was placed on using only those mitochondria exhibiting such respiratory control in all incubations discussed in this study.

Table 1 illustrates the effect of prostaglandins of the E, F, and A series on 11-β hydroxylase activity. E and F series PGs tested inhibit 11-β hydroxylase activity. At the lowest dose (0.01 μg/ml) $PGF_{1\alpha}$ and $PGF_{2\alpha}$ are the most effective inhibitors ($p < .05$) reducing cortisol levels to approximately 50% control. The highest dose (1.0 μg/ml) resulted in values 15-30% control ($p < .01$) after treatment with prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$. Only PGA_1 and PGA_2 stimulate cortisol formation at all three concentrations. PGA_2 is a particularly potent stimulating agent significantly ($p < .001$) increasing cortisol levels to as much as 1300% control. $PGF_{2\alpha}$ (0.01 μg/ml) increases cortisol formation as well, but this effect decreases at higher doses eventually resulting in a 40% reduction in measured cortisol.

No clear dose response is identifiable when all three doses tested are examined collectively. This is due primarily to the overall reduction in cortisol levels noted at the highest dose. At the two lower doses, however, in all the PGs tested except PGE_2 and $PGF_{2\alpha}$ an increase in PG concentration

Table 1. In vitro effect of prostaglandins upon 11-β hydroxylase activity in isolated bovine adrenocortical mitochondria. The standard incubation media consisted of 0.32M sucrose, 2mM MOPS, 20mM KCl, 10mM KH_2PO_4, 5mM $MgCl_2$, 1mM EGTA, 8mM malate, and 1 μM 11-deoxycortisol (pH 7.2). Incubations were performed at 37°C in a final volume of 0.5 ml. Prostaglandins were added as an ethanolic solution in 5 μl aliquots. Reactions were initiated by the addition of 12.5 μg of mitochondrial protein. Values expressed are the mean of three measurements (nmol cortisol/mg mitochondrial protein) ± the standard error.

PROSTAGLANDIN	CONCENTRATION		
	.01 μg/ml	0.1 μg/ml	1.0 μg/ml
PGA_1[a]	37.0 ± 1.5	56.5 ± 5.3	21.5 ± 0.9
PGA_2	219.7 ± 6.8	242.0 ± 13.6	193.0 ± 6.4
PGE_1	14.7 ± 1.5	21.5 ± 0.3	6.6 ± 0.8
PGE_2	14.9 ± 1.1	12.3 ± 0.9	4.6 ± 0.8
$PGF_{1\alpha}$	9.2 ± 0.5	10.3 ± 1.5	4.5 ± 0.3
$PGF_{1\beta}$	16.0 ± 3.0	19.0 ± 0.6	9.9 ± 2.6
$PGF_{2\alpha}$	8.4 ± 1.2	14.4 ± 3.3	2.7 ± 0.3
$PGF_{2\beta}$	36.0 ± 1.0	21.6 ± 0.6	11.6 ± 0.3
[a]Control value	18.5 ± 3.0		

results in an increase in cortisol formation regardless of whether or not the overall effect is stimulatory or inhibitory.

In an effort to determine a mechanism by which PGs alter 11-β hydroxylase activity we examined the interaction between PGs and electron transport in enzymes of the respiratory chain. The pathways for electron transport in steroid 11-β hydroxylation and the respiratory chain are linked (13). Prostaglandins could, therefore, alter steroid hydroxylation by altering the coupling of electron transport to ATP synthesis. Table 2 represents the effect of PGs on succinate supported respiration in bovine adrenocortical mitochondria. Succinate was chosen as the respiratory substrate in these experiments because malate can bypass the respiratory electron transport system utilizing a NADP-dependent malate dehydrogenase (malic enzyme) to support hydroxylation (13). PGE_2 and $PGF_{2\alpha}$ influence respiratory control reducing the ADP:O ratio in the presence of these PGs. These PGs, therefore,

Table 2. Effect of Prostaglandins on succinate supported respiration. Each value is the mean of eight ratios* ± SEM.

PROSTAGLANDIN	CONCENTRATION			
	.01 μg/ml	0.1 μg/ml	1.0 μg/ml	10 μg/ml
PGA_1	1.05 ± .02	.935 ± .53	.943 ± .36	1.03 ± .05
PGA_2	1.14 ± .03	1.09 ± .07	.980 ± .17	1.03 ± .09
PGE_2	.952 ± .35	1.72 ± .07	1.60 ± .01	1.29 ± .10
$PGF_{1\alpha}$	1.04 ± .10	1.24 ± .10	1.00 ± .05	.901 ± .03
$PGF_{2\alpha}$	1.13 ± .15	1.37 ± .02	1.15 ± .06	.917 ± .41

*Ratio: $\frac{\text{ADP:O ratio without PG}}{\text{ADP:O ratio in presence of PG}}$

slightly uncouple oxidative phosphorylation (i.e. increase O_2 uptake when compared with control) in bovine adrenocortical mitochondria. This uncoupling could result from a larger number of reducing equivalents being shunted into the respiratory pathway and away from the hydroxylation pathway. We would expect a reduction in cortisol formation and examination of Table 1 reveals a reduction in cortisol levels in PGE_2 and $PGF_{2\alpha}$ treated mitochondria when compared with control. The other PGs examined reveal little if any effect on succinate supported respiration.

DISCUSSION

Rolland and Chambaz (7) have stated that PGs produce no significant change in 11-β hydroxylase activity which can be detected in their preparations of bovine adrenocortical mitochondria. They do not, however, offer evidence to support this conclusion. We have to the contrary shown that PGs do alter 11-β hydroxylation and that the extent of their effect depends on the particular PG examined.

Penny and coworkers (14) have demonstrated that administration of PGE_1 and PGE_2 to rats *in vivo* results in extensive morphological changes in their adrenocortical mitochondria; changes which suggest a rearrangement of the inner membrane. This information coupled with the knowledge that 11-β

hydroxylase is an enzyme system bound to the inner membrane strongly suggests the mitochondrion as a potential target for PGs generated intracellularly. Our studies demonstrate PG-induced changes in an inner membrane associated process, succinate supported respiration, and indicate that these changes are coupled to changes in 11-β hydroxylase activity. Further, in other tissues indomethacin will inhibit state 3 respiration and β oxidation (15, 16) whereas direct addition of PG's to rat liver mitochondria will increase Ca^{+2} induced respiration (17).

In this study we have characterized the interaction of several of the primary prostaglandins with adrenocortical 11-β hydroxylase and in doing so have complied evidence implicating a direct interaction between prostaglandins and mitochondrial enzymes of the steroid biosynthetic pathway.

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