PROSTAGLANDIN MODULATION OF THE MECHANISM OF ACTH ACTION IN THE HUMAN ADRENAL

Kenneth V. Honn and Walter Chavin
Departments of Radiology and Biology
Wayne State University, Detroit, Michigan 48202, U.S.A.

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Summary: PGE_1 and PGE_2 significantly increased human adrenal cAMP levels in vitro; cortisol output was also increased in a dose related fashion. In contrast, PGF_{1a} and PGF_{2a} depressed adrenal cAMP (except PGF_{2a} at 100 ug/ml). PGF_{1a} and PGF_{2a} depressed cortisol levels at all doses. Indomethacin or 7-oxa-13-prostynoic acid did not affect these parameters. However, when applied in conjunction with ACTH they inhibited or enhanced hormonal action depending upon the temporal sequence of application. The findings indicate that prostaglandins modulate ACTH-adrenocortical cell interaction bidirectionally, initially potentiating and subsequently depressing ACTH stimulated events.

Prostaglandins have been demonstrated to stimulate adrenal steroidogenesis in a number of mammalian species. Initially, such was believed to occur primarily via pituitary ACTH release (1), but subsequently the direct effects of prostaglandins on adrenocortical cAMP (2-4) and steroidogenesis (2,4-6) were demonstrated.

The demonstration of prostaglandin receptors in human and ovine adrenal cell plasma membranes (3) provide direct support to the effects of exogenous prostaglandins in adrenal physiology. ACTH induced E and F type prostaglandin biosynthesis from ^{3}H-arachidonic acid (7) affirm the concept that prostaglandins are a link in the ACTH stimulation of steroidogenesis. However, it is unclear whether prostaglandins function in addition to and/or as an integral part of ACTH-adrenocortical cell interaction. The present study was designed to further illuminate the actions of several prostaglandins on adrenal cAMP and steroidogenesis. Evidence is presented for bidirectional prostaglandin modulation of human adrenal function.

MATERIAL AND METHODS

Six adult human female adrenal glands obtained at surgery were immediately placed in cold (0-4^{o}C) Kreb's Ringer bicarbonate buffer, KRBGA (pH 7.4,

200mg glucose/dl, 0.5% serum albumin fraction V). Glands were diced (2x3 mm) and preincubated (37°C) in KRBGA for 45 min. These dice then were incubated (1ml KRBGA; 37°C; 95%O_2+ 5%CO_2) in a Dubnoff metabolic shaker for 1-32 min. The dice were exposed to prostaglandins E_1, E_2, F_{1a} or F_{2a} at 1,10,100 ug/ml, prostaglandin vehicle (2% ethanol in KRBGA), indomethacin (10 ug/ml), EC-I-148 (7-oxa-13-prostynoic acid; 50 ug/ml), porcine ACTH (100 mIU/ml; chromatographically pure; 150 IU/mg) or KRBGA alone. Further, dice were incubated initially (4 min) in ACTH, indomethacin or EC-I-148 followed by transfer to ACTH plus the appropriate test substance. Adrenal incubates were quenched in liquid nitrogen and analyzed for cAMP by RIA (8). Cortisol secretion into the incubation medium was quantitated by RIA (9). Proteins were determined (10) and the data expressed as pM cAMP or ng cortisol/mg protein, $\bar{X}\pm$SEM. A minimum of four replicates were used per datum point. Data were analyzed by analysis of variance and student t test. Differences were accepted as significant when $p < 0.05$.

Table 1. In Vitro Effects of Prostaglandins E_1, E_2, F_{1a} and F_{2a} Upon Human Adrenocortical cAMP Levels. Incubation Intervals Represent The Earliest Times At Which Maximal Stimulation Or Depression Of cAMP Levels Were Observed.

Incubation Interval (min)	Treatment	pM cAMP /mg protein (Mean ± SEM)
2	KRBGA	5.3 ± 0.2
8	KRBGA	4.4 ± 1.4
16	KRBGA	4.3 ± 2.0
2	PGE_1 1 ug/ml	10.9 ± 3.5*
2	PGE_1 10 ug/ml	17.8 ± 2.5*
2	PGE_1 100 ug/ml	33.0 ± 6.6*
8	PGE_2 1 ug/ml	7.9 ± 3.6
8	PGE_2 10 ug/ml	7.5 ± 4.0
8	PGE_2 100 ug/ml	38.0 ± 7.4*
8	PGF_{1a} 1 ug/ml	1.2 ± 0.8*
8	PGF_{1a} 10 ug/ml	2.6 ± 1.0
8	PGF_{1a} 100 ug/ml	undetectable
8	PGF_{2a} 100 ug/ml	21.0 ± 5.8*
16	PGF_{2a} 1 ug/ml	1.8 ± 0.8
16	PGF_{2a} 10 ug/ml	2.1 ± 0.5

*Statistically different from corresponding control values.

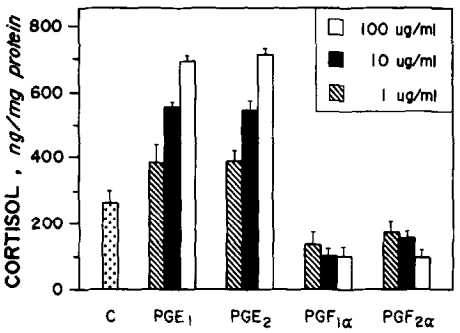

Fig 1 In vitro dose related prostaglandin stimulation (PGE_1, PGE_2) and depression (PGF_{1a}, PGF_{2a}) of cortisol output (32 min) relative to controls (C) by human adrenocortical tissue.

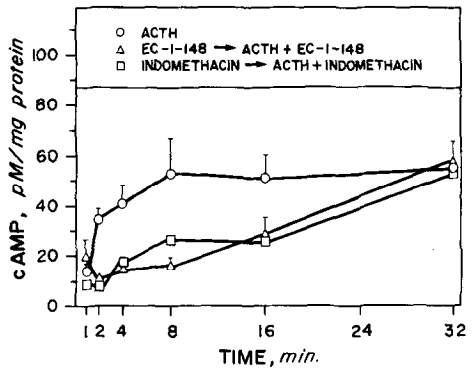

Fig 2 Effects of preincubation (4 min) in either 7-oxa-13-prostynoic acid (EC-I-148) or indomethacin on the subsequent cAMP response to ACTH (100 mIU/ml) compared to the control cAMP response to this ACTH dose in human adrenocortical tissue.

RESULTS

PGE_1 (1,10,100 ug/ml) significantly increased cAMP levels with a peak response occurring within 2 min (Table 1). PGE_2 significantly increased cAMP levels (8 min) at 100 ug/ml; 1,10 ug/ml increased cAMP levels (8 min) 80% and 70% respectively but were not statistically significant. PGF_{1a} (1 ug/ml) significantly depressed cAMP levels (8 min) below controls while 10 ug/ml depressed cAMP levels (8 min) 59% and 100 ug/ml resulted in undetectable cAMP levels (Table 1). Although not statistically significant, PGF_{2a} (1,10 ug/ml) decreased cAMP levels (16 min) 58% and 51%, respectively.

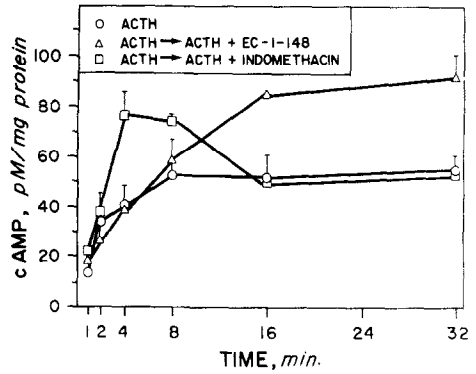

Fig 3 Supramaximal cAMP response of human adrenocortical tissue with indomethacin or 7-oxa-13-prostynoic acid (EC-I-148) in ACTH following preincubation (4 min) in ACTH (100 mIU/ml).

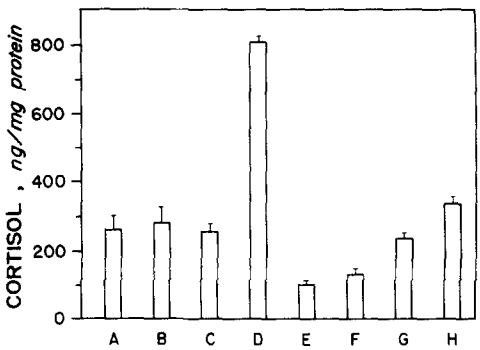

Fig 4 Cortisol output by human adrenocortical tissue following 32 min incubation in:

A. KRBGA
B. 7-oxa-13-prostynoic acid
C. indomethacin
D. ACTH
E. ACTH following 7-oxa-13-prostynoic acid preincubation
F. ACTH following indomethacin preincubation
G. 7-oxa-13-prostynoic acid following ACTH preincubation
H. indomethacin following ACTH preincubation

Interestingly PGF_{2a} (100 ug/ml) significantly increased cAMP levels (8 min) above controls (Table 1). PGE_1 and PGE_2 significantly increased cortisol in a dose related manner, whereas PGF_{1a} and PGF_{2a} significantly depressed cortisol output at all doses studied (Fig 1). The vehicle did not produce any alterations from the control incubations in KRBGA alone in the two parameters evaluated.

Neither indomethacin or EC-I-148 significantly affected cAMP levels. However, pretreatment (4 min) with either test substance followed by incubation in ACTH and the test substance resulted in a significant depression of cAMP levels below that produced by ACTH at 2-16 min (Fig 2). Alternatively, pretreatment (4 min) with ACTH followed by incubation in ACTH and either test substance (Fig 3) resulted in an heightened cAMP response at 4-8 min (indomethacin) or 16-32 min (EC-I-148). Neither indomethacin or EC-I-148 alone significantly altered cortisol output from that of the controls (Fig 4). Cat adrenocortical cell suspensions, however, show augmented corticoid output at this indomethacin concentration (11). Preincubation in either test substance followed by ACTH and the test substance significantly depressed cortisol output below control and ACTH levels. Cortisol secretion after ACTH preincubation followed by ACTH and test substance was significantly lower than ACTH alone but significantly higher (indomethacin) than the controls (Fig 4).

DISCUSSION

The human adrenal is stimulated by the E series prostaglandins to increase both cAMP levels and cortisol output. This agrees with the basic findings in the lower forms studied. The cAMP response appears mediated via activation of adenylate cyclase, but alternate effects of the prostaglandins upon phosphodiesterases, protein kinases, ATPase, etc. also resulting in increased cAMP levels should not be overlooked (12). Time may be an important factor in the understanding of adrenocortical cell prostaglandin interactions. The present data indicate that PGE_1 evoked the maximal cAMP response with considerable rapidity (2 min) compared to ACTH (8 min). The decreased cAMP response time suggests the possibility of direct activation of adenylate cyclase by this prostaglandin to achieve more than a six fold increase in the cyclic nucleotide. PGE_2, on the other hand achieved the cAMP peak response at (8 min) thereby suggesting that alternate mechanisms may be operative in this instance. In addition, both E series prostaglandins stimulated cortisol release on a similar temporal basis despite the temporally dissimilar cAMP response. As a detectable cAMP increase is not always a prerequisite for significant corticosteroid release (13) the possibility of prostaglandin mediated events affecting steroidogenesis without the intervention of cAMP merits consideration.

PGF_{1a} and PGF_{2a} act in a manner antagonistic to that of the E type prostaglandins in that the former decrease both human adrenal cAMP and cortisol output. An inverse relationship between the E and F type prostaglandins also has been suggested in other systems (11). An interesting exception to the aformentioned results is the stimulation of human adrenal cAMP by high levels (100 ug/ml) of PGF_{2a}. This finding is not inconsistent with the hypothesis that E and F prostaglandin are antagonistic, for high levels of PGF_{2a} have been observed to nonspecifically cross react with E prostaglandin receptors producing results characteristic of that group (12). Notably, although PGF_{2a} (100 ug/ml) increased human adrenocortical cAMP levels, corticoid output was depressed, a result consistent with the other doses of F prostaglandins tested. Clearly the prostaglandin mediated cAMP and corticosteroid alterations can be considered separate events. The stimulation of adrenocortical production of both E and F type prostaglandins by ACTH (7) would appear to be paradoxical as the two prostaglandin types have opposing effects. However, considering our experiments which combine ACTH with a prostaglandin inhibitor (indomethacin) or antagonist (7-oxa-13-prostynoic acid) it is clear that the temporal sequence of the interaction produces either cAMP inhibition or a heightened cAMP response. Therefore, both sets of experiments suggest that the ACTH-adrenocortical cell interaction may be modulated by prostaglandins in a bidirectional fashion in that prostaglandins may initially potentiate the ACTH effect but subsequently depress that effect or vice versa. This may occur via a negative feedback by a single type of prostaglandin or an antagonistic interaction between prostaglandins of the E and F series.

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