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# IN VITRO EFFECT OF PROSTAGLANDINS ON CORTICOSTERONE AND ALDOSTERONE PRODUCTION BY FROG INTERRENAL GLAND.

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SUMMARY.

In order to elucidate the role of prostaglandins of the E and F series on adrenal steroidogenesis, we have studied corticosterone and aldosterone production by frog interrenal. Rana ridibunda interrenal dice were perifused with amphibian culture medium for ten hours. Corticosterone and aldosterone concentrations were measured in the effluent perifusate using sensitive and specific radioimmunoassay methods. Perifusion of interrenal fragments with increasing concentrations of $PGE_1$ and $PGE_2$ (ranging from 8.8 nM to 2.8 µM)led to a dose-related increase in both corticosterone and aldosterone biosynthesis, the magnitude of the stimulation being 1.3 fold higher for aldosterone than for corticosterone. High concentrations of $PGF_{2\alpha}$ (2.8 µM) were only responsible for a slight increase in corticosteroid biosynthesis while $PGF_{1\alpha}$ was almost inactive. Indomethacin an inhibitor of prostaglandin biosynthesis caused a marked decrease of spontaneous production of corticosterone (-84%) and aldosterone (-75%) but did not alter the stimulation of steroidogenesis induced by ACTH. From these data, it was concluded that 1) exogenous prostaglandins control corticosteroid production in amphibia ; 2) endogenous prostaglandins are required for spontaneous biosynthesis of corticosteroids ; 3) endogenous prostaglandins are not involved in ACTH-induced steroidogenesis.

The studies which have been designated to investigate the role of prostaglandins (PGs) in the regulation of adrenocortical steroidogenesis have yielded to conflicting results. According to the type of PGs used, to the animals studied and to the technique employed, both inhibition (1), non stimulation (2, 3) and stimulation (4-6) have been reported. Concurrently, controversial results have been obtained with indomethacin (IDM), a potent inhibitor of PG synthesis. Gallant and Brownie (2) have shown that, in vivo, IDM does not

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modify the spontaneous release of corticosterone but inhibits the adrenal response to ACTH, suggesting that PGs are only involved in ACTH-induced steroidogenesis. Conversely, Lowry et al (7) did not find any effect of $PGE_2$ and IDM on ACTH-induced steroidogenesis from isolated rat adrenal cells whereas Flack and Ramwell (4) observed a significant increase in corticosterone synthesis under $PGE_2$ from perifused rat adrenals. In light of these results, it has been inferred that PGs either may not be involved in the control of the adrenal physiology (1, 3, 7) or may act on adrenocortical steroidogenesis through different mechanisms including i) direct alteration of specific enzymes of the corticosteroid biosynthetic pathway (8) ; ii) stimulation of cyclic AMP generation (9, 10) ; and iii) allosteric control of ACTH-binding at the adrenal receptor level (2). In the present study, we have decided to explore the action of PGs on corticosterone and aldosterone production from perifused amphibian interrenal (adrenal) glands. The data reported in this paper demonstrate that the PGs of the E pathway stimulate spontaneous steroidogenesis but are not required for ACTH induced corticosteroid production.

## MATERIALS AND METHODS

Preparation of the perifusion chamber. For all studies, male frogs (Rana ridibunda Pallas) of 30-40 g body weight were used. The animals were kept at least one week in glass tanks with circulating tap water before experimentation. The method of dissection of the interrenal (adrenal) glands and the procedure for perifusion have been described elsewhere (11). Briefly, for each experiment, 9 frogs were killed between 0800 - 0900 h by decapitation. The kidneys were quickly placed in 300 µl Amphibian Culture Medium (ACM) prepared according to Wolf and Quimby (12) by Eurobio (Paris). The interrenal glands were dissected, minced in small dice (< 0.1 $mm^3$ each) and pre-incubated for 15 min in 5 ml ACM, gassed with moistened $O_2$ : $CO_2$/95 : 5. Then the interrenal dice were mixed with biogel $P_2$ and transferred into a siliconized glass column (0.9 x 2.5 cm). The temperature of the column was kept constant at 24°C throughout the experiment. Glands were perifused either with ACM or with test substances dissolved in ACM. The culture medium was delivered to the perifusion chamber by a peristaltic pump at a constant flow rate of 350 µl/min. After an initial 30 min stabilization period, the effluent perifusate was collected every 5 min in tubes and immediately frozen.

Steroids radioimmunoassays. Corticosterone and aldosterone concentrations were determined by RIA, in all fractions collected, without prior extraction. Details concerning corticosterone (13) and aldosterone (14,15) radioimmunoassays have been described elsewhere . For both assays, the within-assay coefficient of variability was lower than 2% and the between-assay coefficient of variability was 3%. The specificity of the antibodies

has been evaluated by determining their cross-reactivities with 22 different steroids and various compounds. Corticosterone antibodies exhibited significant cross-reactions with progesterone (19%), 11-deoxycorticosterone (17%) and 21-deoxycortisol (8%). Lower cross-reactivity (<4%) was observed between corticosterone antibodies and other steroids tested, mainly aldosterone (0.63%) and testosterone (1.8%). The aldosterone antibodies showed very weak cross-reactions (<0.005%) with all related compounds

Sephadex LH-20 chromatography. In order to ensure that corticosterone or aldosterone were the only steroids that encounted for corticosterone or aldosterone-like immunoreactivity in the effluent medium, a number of perifusate fractions have been submitted to gel chromatography. The perifused samples were extracted with 12 volumes of dichloromethane and chromatographed over a Sephadex LH-20 column (0.9 x 40 cm), in the solvent system dichloromethane:methanol/98:2 (Vol., Vol.). Sixty fractions (1.4 ml each) were collected and evaporated. In each fraction, corticosterone and aldosterone concentrations were radioimmunoassayed as described above.

Secretagogues (test substances). Preliminary experiments were designed to verify that ethanol concentrations up to 1% were totally devoid of effect on corticosteroid production. Prostaglandins and indomethacin (secretagogues) were dissolved in ethanol and the final dilutions were made up in ACM just before use, so that ethanol concentration was 0.25%. All other solutions contained 0.25% ethanol. At the concentrations studied, none of the secretagogues interfered in the corticosterone and aldosterone radioimmunoassays.

## RESULTS

Sephadex LH-20 gel chromatography. Figure 1 shows a typical elution profile of a dichloromethane extract of the effluent perifusate. A single peak of aldosterone co-eluted exactly in the same position as unlabeled or $^{3}$H-labeled aldosterone. Similarly a single peak of corticosterone co-migrating with unlabeled and $^{3}$H-labeled corticosterone was observed.

Effect of prostaglandins on spontaneous corticosteroid biosynthesis. In figure 2 are represented two typical perifusion profiles observed during $PGE_1$ administration. A 30 min perifusion in the presence of $PGE_1$, at concentrations ranging from 8.85 nM to 2.8 µM induced a dose-related increase in corticosterone release (Fig. 2 a). The lowest concentration induced a significant increase in corticosterone output (+ 29% : peak height vs spontaneous secretion). A 3-fold increase in corticosterone output was observed with the highest concentration of $PGE_1$ tested (2.8 µM). The lag period was less than 5 min and the duration of the response ranged from 60 to 90 min. Similarly responses in aldosterone output were attained although 1) the minimum effective dose of $PGE_1$ was 3-fold higher than for corticosterone and 2) a plateau was reached with $PGE_1$ concentrations higher than 0.28 µM. The dynamics of the response was almost

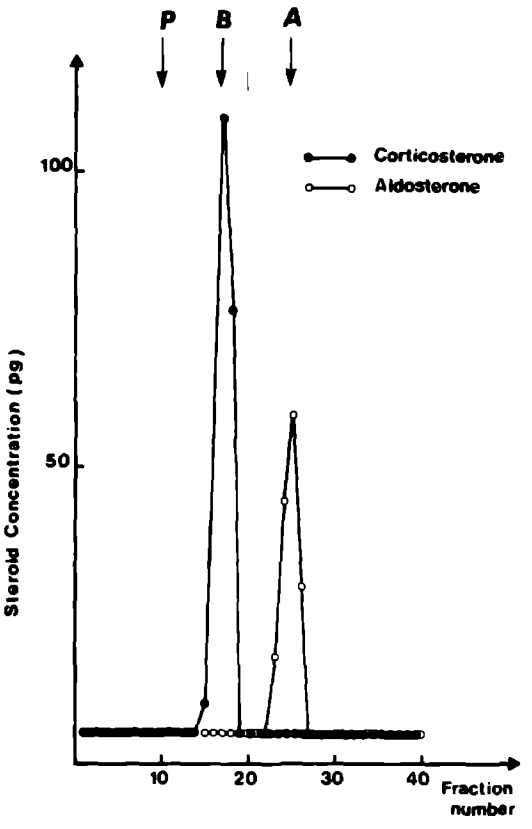


*Figure 1: Sephadex LH-20 elution pattern of a dichloromethane extract of the effluent perifusate. Corticosterone and aldosterone - like materials were radioimmunoassayed as described in text. The column was calibrated (arrows) with synthetic unlabeled and labeled progesterone (P), corticosterone (B) and aldosterone (A).*

identical to that observed for corticosterone : the lag period was less than 5 min and the duration of the response ranged from 60 to 90 min.

A series of experiments similar to those represented in figures 2a and 2b were conducted with $PGE_2$, $PGF_{1\alpha}$ and $PGF_{2\alpha}$. The corresponding dose-response curves are represented in figure 3. The values are expressed as the maximum corticosteroid level (peak height) vs basal corticosteroid output. All four primary PGs were able to elicit corticosteroid production but PG of the E series were much more potent stimulators of corticosteroid production than PGFs. For the different prostaglandins studied the following order of potency was found : $PGE_1 > PGE_2 >> PGF_{2\alpha} > PGF_{1\alpha}$.

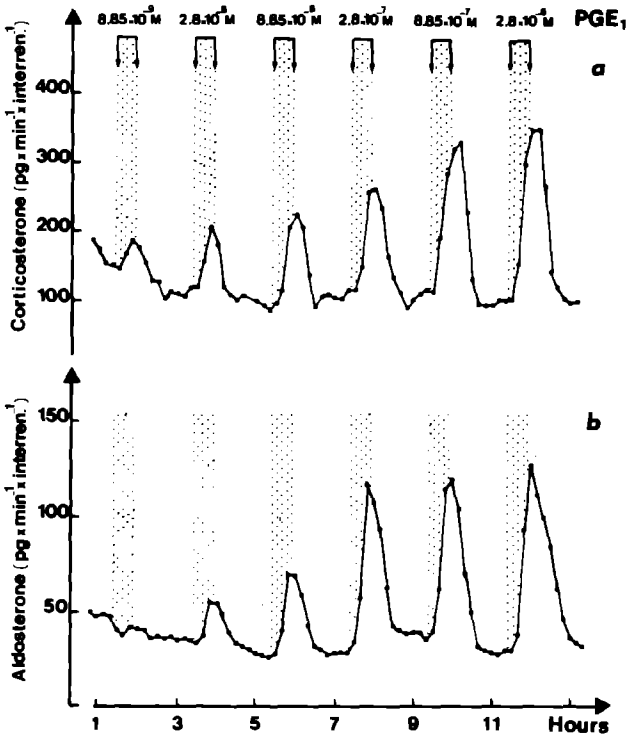


*Figure 2: Effect of increasing doses of $PGE_1$ ($8.8 \times 10^{-9}$M to $2.8 \times 10^{-6}$M) on corticosterone (a) and aldosterone (b) output. The interrenal fragments were perifused for 30 min with graded concentrations of $PGE_1$. The system was allowed to run 90 min with ACM alone between two consecutive doses of $PGE_1$. Each point represents the mean corticosteroid concentration of two consecutive fractions collected during 5 minutes.*

Effect of indomethacin on spontaneous and ACTH-induced corticosteroid biosynthesis. Figure 4 represents the effects of ACTH alone or during prolonged indomethacin infusion on corticosterone (a) and aldosterone (b) production. As previously shown (11,14), nanomolar concentrations of synthetic ACTH induce, in the present model, a significant increase in both corticosterone and aldosterone biosynthesis. Conversely, indomethacin ($5 \times 10^{-6}$M) was responsible for a decrease in corticosterone (-84%) and aldosterone (- 75%) output. In spite of this drastic inhibitory effect, indomethacin did not affect the corticosterone and aldosterone responses to ACTH.

## DISCUSSION

The contribution of prostaglandins in the spontaneous production of corticosteroids and/or in the molecular mechanism involved in the action of

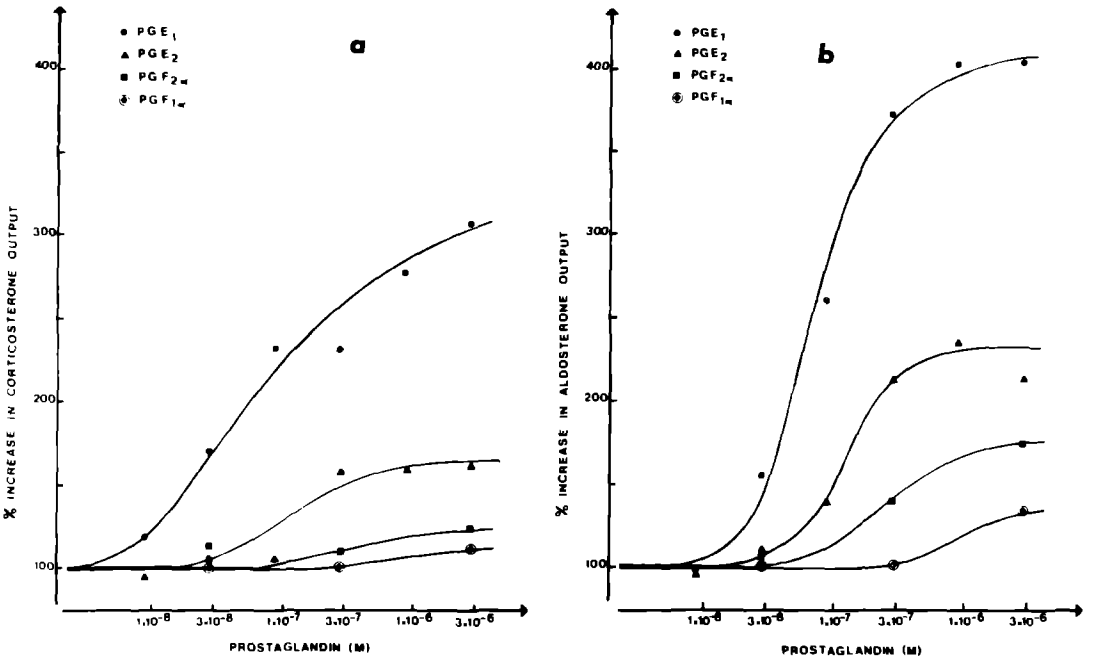


*Figure 3: Semilogarithmic plot comparing the effect of prostaglandins of the E and F series on corticosterone (a) and aldosterone (b) production. All experimental values were calculated from data similar to those represented in Figure 2. The mean corticosteroid concentration in 4 consecutive fractions collected during and after perifusion with PGs (peak height) were compared to the mean corticosteroid levels observed just prior to the infusion of each dose of PGs (spontaneous production).*

ACTH continues to be a challenging problem. Our present results show that PGs of the E pathway exert a direct stimulation of both glucocorticoid and mineralocorticoid productions by frog adrenal gland in vitro. These results are in agreement with those obtained with human adrenal dice (10) and rat capsulated adrenals (5) whereas other investigators, using acutely dispersed rat adrenal cells were unable to demonstrate a direct effect of exogenous or endogenous PGs on steroid biosynthesis (3 7). The reason for this difference has not yet been elucidated but it seems likely that the tryptic digestion of the cell coat may alter prostaglandin receptors in those cells.

Our findings that $PGE_1$ is the most potent primary prostaglandin are in accordance with previous studies (9,10) except that frog adrenal dice are 100 to 1000 fold more sensitive than human or rat preparations. However, it

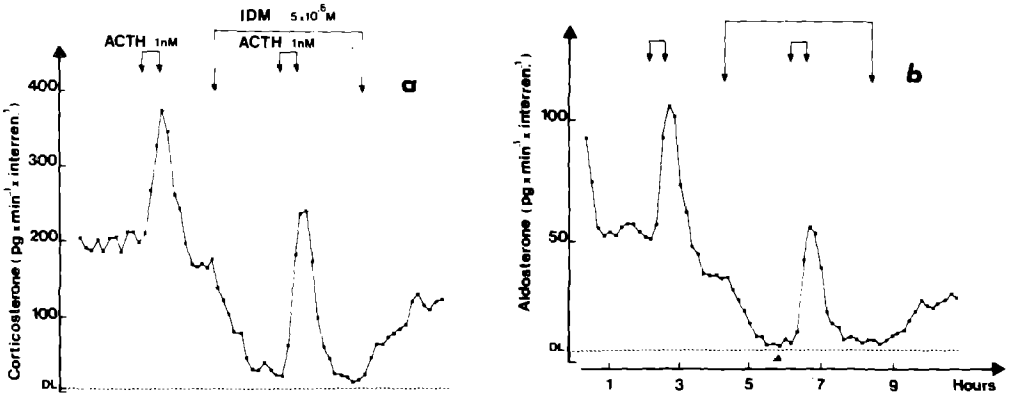


*Figure 4: Effect of ACTH (1 X $10^{-9}$M) alone and during the infusion of indomethacin (IDM ; 5 X $10^{-6}$M) on corticosterone(a) and aldosterone(b) biosynthesis.*

must be mentioned that Flack et al. (16) have reported that $PGE_2$ was more potent than $PGE_1$.

The advantage of the perifusion model is to provide valuable information concerning the time-course effect of prostaglandins on adrenal steroidogenesis. Detailed examination of the perifusion pattern showed that the kinetics of the response of the glands was exactly the same as previously found for ACTH (14) : the latent period was 5 min ; the maximum stimulation was achieved 30 to 50 min after the beginning of the infusion of the compound, independently of the secretagogue concentration ; baseline level was reached within 30 to 60 min after the passage of the drug. Worthy of note is the total discrepancy between our results and those reported by Chavin et al. (17), who found in frog that 1) $PGE_1$ stimulated corticosterone but failed to increase significantly aldosterone biosynthesis ; 2) $PGE_2$ was devoid of effect on both corticosterone and aldosterone biosynthesis ; 3) $PGF_{1\alpha}$ inhibited aldosterone output.

In the second part of our study, we took opportunity of the availability of our dynamic model to gain insight into the role of endogenous prostaglandins in spontaneous and corticotropin-stimulated corticosteroid biosynthesis. Since indomethacin significantly inhibited the baseline level of both corticosterone and aldosterone, it was evident that prostaglandin synthesis within adrenal

cells is essential for the maintenance of spontaneous corticosteroidogenesis. Conversely, it appeared that indomethacin did not impair the response of adrenal gland to ACTH. Thus, the data presented herein do not support the concept proposed by other authors (18) that the stimulation of corticosteroid biosynthesis induced by ACTH is mediated by an increase in prostaglandin synthesis.

Our findings high lights one important difference between the effects of indomethacin in vivo and in vitro. When administered in vivo, indomethacin did not modify plasma corticosterone concentration in rat and significantly inhibited the increased corticosterone biosynthesis induced by ACTH (19). The reasons for this difference are not apparent, but it should be pointed out that the very high doses of $PGE_2$ employed for the in vivo study (200 µg/rat) must affect adrenal and renal blood flow and alter corticosteroid production. Furthermore in preliminary unpublished experiments, we have found that, in vitro, high concentrations of prostaglandins (up to $2.8 \times 10^{-4}$M) were 3 to 7 times less active than a lower concentration ($2.8 \times 10^{-6}$M).

Further investigations with our dynamic perifusion model are expected to provide additional insight into the mechanism of action of prostaglandins at the adrenal level.

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