Role of Prostaglandins in Angiotensin-Induced Steroidogenesis
Absence of an Effect by Prostaglandin E₂

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SUMMARY Recently, we have found that the prostaglandin synthesis inhibitor, indomethacin, reduced basal and angiotensin stimulated aldosterone release. To further test the possibility that prostaglandins (PGs) function as mediators of adrenal steroidogenesis, we examined the release of aldosterone, PGE₂ and PGF₃₀ under basal and stimulated conditions in isolated adrenal capsular cells in vitro. Angiotensin II and III caused a dose-related increase in aldosterone release without significantly altering the release of PGE₂ or PGF₃₀. Indomethacin inhibited basal, angiotensin II, and angiotensin III-induced steroidogenesis by 40%, 15%, and 52% respectively. Additionally, it inhibited the release of PGE₂ by 60% in the control and angiotensin-treated cells. In indomethacin-treated cells, PGE₂ stimulated aldosterone release in supraphysiological doses; however, its steroidogenic effect was not additive with angiotensin II. The prostaglandin precursor, arachidonic acid, increased the adrenal synthesis of PGE₂ and PGF₃₀ in a dose-related manner without altering the synthesis of aldosterone. Similarly, the prostaglandin endoperoxide PGH₂ increased the synthesis of PGE₂ by 250-fold, yet failed to alter aldosterone synthesis. These findings indicate that PGE₂ does not mediate or modulate basal or angiotensin-stimulated steroidogenesis. Furthermore, it would appear that indomethacin may inhibit adrenal steroidogenesis via a mechanism other than inhibition of prostaglandin synthesis.

KEY WORDS • indomethacin • arachidonic acid • prostaglandin H₂ • prostaglandin F₃₀ • angiotensin II • angiotensin III

RECENTLY, we have found that the prostaglandin synthesis inhibitor indomethacin inhibited the release of aldosterone by angiotensin II and III in normal and sodium-depleted conscious rats and in rat adrenal cell suspensions. Another prostaglandin synthesis inhibitor, meclofenamate, inhibited angiotensin III but not angiotensin II-induced steroidogenesis. Since both drugs simultaneously inhibited the urinary excretion of prostaglandin E₂ (PGE₂) and prostaglandin F₃₀ (PGF₃₀) reduced the adrenal prostaglandin content and reduced the adrenal conversion of ¹⁴C-arachidonic acid to ¹⁴C-PGE₂ and ¹⁴C-PGF₃₀, it was suggested that adrenal prostaglandins mediate a portion of the steroidogenic effects of the angiotensins. This contention was also suggested by the observations of Saruta and Kaplan that PGE₁ stimulated the release of aldosterone from adrenal cortical slices, an effect that was additive with angiotensin II. Subsequent studies confirmed the steroidogenic effects of PGE₂ and PGF₂α in vitro but not in vivo; however, it should be mentioned that in each of the in vitro studies supraphysiological doses of the prostaglandins were required for the release of aldosterone.

Our present studies were designed to further test the possibility that angiotensin stimulates the adrenal production of PGE₂ and that this PGE₂ augments the steroidogenic activity of the peptide. To accomplish this, we examined the effects of angiotensin II and III on the release of aldosterone, PGE₂, and PGF₃₀ in adrenal cell suspensions. Also, the effects of the precursors of these prostaglandins, arachidonic acid and prostaglandin cyclic endoperoxide (PGH₂), on the release of aldosterone were determined.

Methods

Male Sprague-Dawley rats (225–300 g; Simonsen Laboratories) were used in these studies. The rats were maintained on a standard Wayne Rat Chow diet containing 142 mEq/kg of sodium and 290 mEq/kg of potassium and tap water ad libitum.

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Adrenal cell suspensions were prepared by the method of Sarstedt et al.1 with several modifications.1 Rats were killed by decapitation, and their adrenal glands were removed and placed in potassium-free medium 199. The adrenals were separated from the adhering fat, and the capsules removed. The capsular tissue was minced and incubated in potassium-free medium 199 containing 0.5 mg/ml of trypsin (Sigma) for 15 minutes in a Dubnoff metabolic shaker at 37°C under an atmosphere of 95% O₂ and 5% CO₂ with 60 rpm agitation. After 30 and 60 minutes of incubation, the cells were dispersed with potassium-free medium 199 containing 2 mg/ml of bovine serum albumin. In the studies that followed, the bovine serum albumin was omitted from the final incubation buffer since it is known to bind fatty acids and convert PGH₁ to PGD₂.* The adrenal cells were counted with a hemocytometer, and the number of viable cells determined by exclusion staining of trypan blue. Using this method, we obtained approximately 300,000 viable cells per adrenal capsule, and the viability of the cells was approximately 94%. We then placed 0.5 ml containing 500,000 to 700,000 cells in a Dubnoff metabolic shaker at 37°C under an atmosphere of 95% O₂ and 5% CO₂. All compounds were added in a constant volume of 10 μl, and an equal volume of their vehicle was added to the control tubes. Following these additions, the cells were incubated for 1 hour. At the end of the incubation period, the cell suspensions were centrifuged and the supernatant assayed for aldosterone by direct radioimmunoassay. The results were expressed as nanograms (ng) of aldosterone/10⁶ cells/hr. The remainder of the supernatants was combined from two incubation tubes and assayed for PGE₁ and PGF₂α by radioimmunoassay.

Prostaglandin E₁ and F₂α were measured by the method of Dray et al.8 with several modifications. The PGE₁ and PGF₂α antisera were produced in our laboratory in rabbits immunized against a prostaglandin-thyroglobulin conjugate. The conjugate was prepared by the method of Jaffe and Behrman,10 mixed with Freund's complete adjuvant (Grand Island Biologicals), and injected monthly in multiple subcutaneous sites in rabbits. The cross reactivities of the antibodies with known prostaglandins are listed in Table 1.

The assay consisted of extracting 1 ml of incubation media (containing 1000 cpm of ³H-PGE₁ and ³H-PGF₂α) with 10 ml of ethyl acetate:cyclohexane (50:50) after acidification to pH 3.0 with glacial acetic acid. The organic phase was then removed and evaporated to dryness at 30°C under nitrogen. The extract was reconstituted in a benzene:ethyl acetate:methanol mixture (60:40:10) and placed on an 0.8 x 10 cm silicic acid column (Sigma, SIL-B200). The prostaglandins were then eluted with solvents of increasing polarity with PGA₁, PGB₁, and PGB₂ in the first fraction, PGE₁ in the second, and PGF₂α in the third (Fig. 1). These column eluates were dried at 30°C under nitrogen and reconstituted in 1.0 ml of phosphate-buffered saline containing 0.1% polyvinylpyrrolidone. Radioimmunoassay was performed by adding 0.1 ml of the unknown to 3000 cpm of ³H-PGE₁ or ³H-PGF₂α and 0.1 ml of the prostaglandin antisera (titer 1:8000 for PGE₁ and 1:30,000 for PGF₂α). After incubating overnight at 4°C, the bound and free prostaglandins were separated by the addition of dextran-coated charcoal. The bound counts were assayed for PGE₁ and PGF₂α to by radioimmunoassay.

### Table 1. Cross-Reactivity of Prostaglandin E₁ and F₂α Antisera with Known Prostaglandins

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Anti-PGE₁ (1:8,000)</th>
<th>Anti-PGF₂α (1:30,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEA</td>
<td>0.30</td>
<td>0.01</td>
</tr>
<tr>
<td>PGB₂</td>
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<td>0.01</td>
</tr>
<tr>
<td>PGE₁</td>
<td>0.10</td>
<td>1.00</td>
</tr>
<tr>
<td>PGE₂</td>
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<td>0.27</td>
</tr>
<tr>
<td>PGE₃</td>
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<td>0.30</td>
</tr>
<tr>
<td>15-keto-PGE₁</td>
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<td></td>
</tr>
<tr>
<td>PGE₂α</td>
<td>0.70</td>
<td>100.00</td>
</tr>
<tr>
<td>PGE₃α</td>
<td>0.01</td>
<td>100.00</td>
</tr>
<tr>
<td>15-keto-PGF₂α</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>15-keto-PGF₂α</td>
<td>0.20</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 1.** Separation of ³H-prostaglandin A₁, E₁, and F₂α by silicic acid (Sigma, SIL-200B) column chromatography.
determined by liquid scintillation spectrometry (Beckman Instruments), corrected for recoveries, and the results expressed as pg/10^6 cells/hr. The sensitivity of the assay was < 5 pg/tube and the recoveries averaged 85%. Also, the interassay and intraassay variability was 9% and 6% respectively.

Since incubation of the cells with ^3H-PGE_2 or ^3H-PGF_2α for periods of 1 and 2 hours failed to demonstrate metabolism or degradation of the prostaglandins, the levels of PGE_2 and PGF_2α measured in the incubation media must reflect adrenal cell synthesis and release only.

Prostaglandin H_2 was biosynthesized by the method of Green et al. using ram seminal vesicle microsomes. The product was identified as PGH_2 by the following criteria: 1) treatment with stannous chloride converted it to a compound that behaved as PGF_2α by chromatographic and immunoassay methods; 2) treatment with bovine serum albumin converted it to a compound with the chromatographic properties of PGD_2; and 3) when added to human platelets, it was converted to immunoreactive thromboxane B_2. The PGH_2 was stored at -20°C in dry diethyl ether. This ethereal solution was added to the adrenal cells during incubation at 37°C, and the diethyl ether vehicle was added to the control cells.

Statistical analyses were performed by analysis of variance and student's t test when comparing groups and by analysis of covariance when comparing dose-response curves.

Results

The effects of angiotensin II and III on the release of aldosterone and prostaglandins from adrenal cells is illustrated in figures 2 and 3. Both peptides caused a dose-related increase in aldosterone release without significantly altering the release of PGE_2 or PGF_2α. At the 10 pmoles dose of angiotensin II, the PGE_2 concentration in the medium was significantly elevated; however, with a higher dose, the PGE_2 concentration returned to control levels.

Indomethacin was found to inhibit basal and angiotensin II-induced aldosterone release by 40% (p < 0.001) and 15% (p < 0.05) respectively (fig. 4). While angiotensin II failed to alter PGE_2 release in the dose tested, indomethacin reduced the release of PGE_2 into the medium by 60% in both control and angiotensin II treated cells. Similar results were obtained with angiotensin III with indomethacin inhibiting angiotensin III-stimulated steroidogenesis by 52% (p < 0.01) and PGE_2 release by 60% (p < 0.05) (fig. 5). When figures 4 and 5 are compared, it is clear that indomethacin was more effective in inhibiting angiotensin III-induced steroidogenesis when compared with angiotensin II despite similar reductions in PGE_2 release.

While angiotensin II did not release PGE_2, it was possible that basal amounts of PGE_2 might "prime" or sensitize the adrenal cortex to angiotensin II and that such an action could explain the inhibitory effects of indomethacin. However, when the effect of angiotensin II on aldosterone release was examined in the presence and absence of PGE_2 in indomethacin-treated cells, similar dose responses were observed in both groups indicating a lack of sensitization by PGE_2 (fig. 6). Furthermore, the effects of angiotensin II and PGE_2 were additive only at the lowest dose of angiotensin II tested.

**Figure 2. Effect of angiotensin II on the release of aldosterone (left) and PGE_2 and PGF_2α (right) in rat adrenal cell suspensions. (Each point represents the mean ± SEM.) *p < 0.05; **p < 0.01, ***p < 0.001 compared with control.**
FIGURE 3. Effect of angiotensin III on the release of aldosterone (left) and PGE_2 and PGF_2α (right) in rat adrenal cell suspensions. *p < 0.05; **p < 0.01; ***p < 0.001 compared with control.

FIGURE 4. Effect of indomethacin on angiotensin II-induced aldosterone release and prostaglandin release in rat adrenal cell suspensions. Each value represents the mean ± SEM. Statistical significance is indicated in the brackets connecting the compared bars.

FIGURE 5. Effect of indomethacin on angiotensin III-induced aldosterone release and prostaglandin release in rat adrenal cell suspensions. Each value represents the mean ± SEM. Statistical significance is indicated in the brackets connecting the compared bars.
To further examine the role of prostaglandins in steroidogenesis, the effects of the fatty acid precursor of PGE₂, arachidonic acid, was tested (fig. 7). While arachidonic acid caused a dose-related increase in PGE₂ and PGF₂α release, the fatty acid failed to alter the release of aldosterone. Similarly, the cyclic endoperoxide intermediate, PGH₂, increased the adrenal PGE₂ production by 230-fold, yet did not alter steroidogenesis (fig. 8).

**Discussion**

Our present experiments indicate that prostaglandin E₂ does not function as a mediator of adrenal steroidogenesis in physiologically relevant doses. While adrenal cortical cells are capable of synthesizing PGE₂ from both arachidonic acid and PGH₂, aldosterone release is not stimulated by these prostaglandin E₂ precursors. Other investigators have reported a dose-related increase in aldosterone release in vitro with prostaglandin E₂ and E₃; however, doses in the microgram range were required for steroidogenic activity. Thus, it appears that prostaglandin E₂ is unable to stimulate aldosterone
secretion in concentrations that the adrenal is capable of synthesizing.

Angiotensin II and III release PGE₃ from the kidney and mesenteric vasculature; however, we could detect no change in the release of PGE₃ from adrenal cortical cells by steroidogenic doses of these peptides. Since adrenal cells contain the cyclooxygenase and isomerase necessary for the synthesis of PGE₃, the failure of angiotensin to release the prostaglandin must be due to a failure of the peptides to release arachidonic acid from its phospholipid or cholesterol ester stores. This lack of stimulation of the angiotensin-sensitive phospholipase could be due to the presence of glucocorticoids in the incubation media since these steroids are known to inhibit phospholipase A₂. However, when steroid synthesis is blocked by aminogluthimide, angiotensin II still fails to stimulate the release of PGE₃ from adrenal cells (M. J. Dunn, personal communication). Thus, the mechanism of adrenal insensitivity to angiotensin is unknown.

As in our previous studies, indomethacin inhibited basal, angiotensin II, and angiotensin III-stimulated aldosterone release as well as inhibiting the adrenal synthesis of PGE₃ by 60%. Interestingly, indomethacin was more effective in inhibiting angiotensin III-induced steroidogenesis than that stimulated by angiotensin II; however, the reason for this difference is unknown. Indomethacin has also been found to inhibit angiotensin-induced steroidogenesis in normal subjects but not in patients with post-malignant hypertension. This 60% decrease in basal prostaglandin synthesis with indomethacin may be interpreted to explain the observed decreases in basal and angiotensin-stimulated aldosterone release. However, since a dose of PGE₃ (100 ng) that was 250 times greater than the basal adrenal cell production was required to stimulate aldosterone production twofold in indomethacin-treated adrenal cells, this would not appear to be the case. Furthermore, we could not demonstrate that this dose of PGE₃ was additive with angiotensin II on steroidogenesis or that it sensitized the adrenal to the actions of angiotensin II.

These findings suggest that indomethacin inhibits aldosterone release by an action independent of prostaglandin synthesis inhibition. Alternatively, indomethacin may inhibit angiotensin-steroidogenesis by blocking the synthesis of an arachidonic acid metabolite other than prostaglandin E₂. The most probable prostaglandin candidate would appear to be prostacyclin, PG₁₂. In this regard, Ellis et al. found that PG₁₂ was 100–1000 times more potent than PGE₃ in stimulating the release of corticosterone from adrenal cortical cells. However, this latter possibility would appear unlikely in view of our findings that steroidogenesis is not stimulated by either arachidonic acid or PGH₁.

In summary, prostaglandin E₂ does not appear to mediate or modulate basal or angiotensin-stimulated steroidogenesis. This is based on the observations that angiotensin II and III do not stimulate prostaglandin synthesis in steroidogenic doses, that supraphysiological doses of PGE₃ are required for steroidogenesis, and that arachidonic acid and PGH₁ fail to stimulate steroidogenesis even though prostaglandin synthesis is increased.

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