Prostaglandin E₂-Induced Aldosterone Release Is Mediated by an EP₂ Receptor

Stephen Csukas, Craig J. Hanke, David Rewolinski, William B. Campbell

Abstract—Prostaglandin E₂ (PGE₂) is an endogenous hormone of adrenal zona glomerulosa cells and is released in response to stimulation by agonists such as angiotensin II (Ang II). It stimulates the release of aldosterone from cultured bovine adrenal zona glomerulosa cells. These studies were designed to determine whether this steroidogenic effect of PGE₂ was mediated by an EP₁, EP₂, or EP₃ receptor. Prostaglandin E₂ and 11-deoxy PGF₁α, an EP₂-selective agonist, stimulated aldosterone release in a concentration-related manner with an ED₅₀ of 90 nmol/L for PGE₂ and 2 μmol/L for 11-deoxy PGF₁α. The maximal effect of PGE₂ was less than that of angiotensin II. 17-Phenyl trinor PGE₂, an EP₁-selective agonist, required concentrations of 100 μmol/L to stimulate aldosterone release and sulprostone, an EP₁/EP₃-selective agonist, failed to alter aldosterone release. The EP₁-selective antagonist SC19220 failed to alter basal or PGE₂-stimulated aldosterone release over a range of concentrations. PGE₂ and 11-deoxy PGF₁α also stimulated an increase in both intracellular and extracellular cAMP. This increase was time- and concentration-related. The ED₅₀ for PGE₂ was 9.8 μmol/L. 17-Phenyl trinor PGE₂ and sulprostone were without effect. Using fura-2 loaded cells, PGE₂ (2 μmol/L), dibutyryl cAMP (2 mmol/L), and Ang II (2 μmol/L) increased intracellular calcium over basal concentrations by 5.5-fold, 3-fold, and 6.2-fold, respectively. Like PGE₂, dibutyryl cAMP also stimulated aldosterone release. PGE₂- and dibutyryl cAMP-induced aldosterone release were blocked by the calcium channel inhibitor diltiazem. These studies indicate that PGE₂ is a potent stimulus for aldosterone release and that the effect is mediated by EP₂ receptors. Both CAMP and calcium appear to mediate the steroidogenic effect of PGE₂ and calcium seems to be distal to cAMP. (Hypertension. 1998;31:575-581.)

Key Words: zona glomerulosa ■ cyclic AMP ■ calcium ■ receptors, prostanooid ■ angiotensin II

There are five classes of prostanoid (P) receptors designated as EP, FP, DP, IP, and TP, corresponding to their naturally occurring agonists, prostaglandin E₂, prostaglandin F₃α, prostaglandin D₂, prostaglandin I₂, and thromboxane A₂, respectively. Synthetic and natural analogues of these prostaglandins also exist that possess selectivity at these five classes of receptors. The EP receptor has been subclassified into three subtypes: EP₁, EP₂, and EP₃. Based on the analysis of Coleman et al, Eglin and Whiting, and Muallem et al, we know that prostanoid receptors differ in the second messengers that mediate their biological effects. Agonists acting on the EP₁, EP₂, or TP receptors stimulate the IP/DAG pathway and exert their effects through an increase in intracellular calcium. The EP₂, DP, or IP receptor agonists stimulate adenylyl cyclase and the accumulation of cAMP. Finally, EP₂ receptor activation may increase IP₃/DAG formation or inhibit adenylyl cyclase.

There is considerable evidence that metabolites of arachidonic acid are involved in the regulation of aldosterone release. We and others have found that PGE₂ stimulates the release of aldosterone in nanomolar concentrations, whereas PGI₂ is without effect. This effect of PGE₂ is due to the prostanoid increasing the conversion of cholesterol to pregnenolone, the early step of aldosterone biosynthesis. The effects of PGI₂ are less clear, possibly due to its instability in physiological solutions. Matsuoka et al and Swartz et al found that PGI₂ did not change aldosterone release; however, PGE₂ also failed to alter steroidogenesis in concentrations up to 100 mmol/L. In contrast, we found that PGI₂ stimulated aldosterone release if added at frequent intervals during the incubation. Using radioligand binding methods, Karaplis and Powell found that adrenal cortical microsomes possessed binding sites for PGE₂. There have been no functional or biochemical studies to characterize the receptor subtype that mediates these adrenal cortical effects of PGE₂. In the present study, cultured bovine adrenal zona glomerulosa cells were examined to determine whether an EP receptor is present, and whether its stimulation is linked to aldosterone release. An examination of second messenger generation and aldosterone production in response to several EP receptor agonists indicated that PGE₂ promotes the release of aldosterone through an EP₂ receptor.

Methods

Tissue Recovery and Culture

Bovine adrenal glands from five or six cows were obtained from a local abattoir on ice. As previously reported and briefly described here,
Selected Abbreviations and Acronyms

ACTH = corticotropin  
BSA = bovine serum albumin  
DAG = diacylglycerol  
DP = prostaglandin D_2  
EBSS = Earle’s balanced salt solution  
ELISA = enzyme-linked immunosorbent assay  
EP = prostaglandin E_2  
FP = prostaglandin F_2  
IP = prostaglandin I_2  
IP_3 = inositol triphosphate  
P = prostanoid  
PG = prostaglandin  
TP = thromboxane A_2.

glands were trimmed of fat and bisected. A Studie–Riggs microtome (Thomas Scientific) was used to cut a 500 μm slice from the outer surface of the gland. This capsular tissue slice was used to prepare zona glomerulosa cells. Adherent cells from the inner cortical zones (zona fasciculata-reticularis) were removed by scraping the surface. Slices of zona glomerulosa tissue were resuspended in a digestion buffer of EBSS containing 25 mmol/L HEPES, collagenase (1.8 mg/mL), hyaluronidase (0.75 mg/mL), dispase (1 mg/mL), fatty acid–free BSA (1 mg/mL), DNase (0.2 mg/mL), penicillin (500 U/mL), and streptomycin (500 μg/mL). The slices were incubated in a Haake stirring circulator water bath at 37°C for 25 minutes with gentle agitation. Slices were dispersed by repeated pipetting through a wide-bore 10 mL pipette, and the suspended cells were centrifuged.

At confluence, each culture well was washed twice with 1 mL modified Ham’s F-12 media supplemented with 14 mmol/L NaCl and 14 mmol/L NaHCO_3, inhibitors, and antioxidants. Cells were plated at a density of 2 to 3 × 10^5 cells per well in 24-well tissue culture–treated plates. Cell viability was ascertained by exclusion staining with trypan blue. Cells were maintained at 37°C in 5% air/5% CO_2. Cells were used on reaching confluence, typically after 3 to 4 days in culture. Based on light microscopy, the purity of zona glomerulosa cells was approximately 95%. Additionally, cortisol release from these cells was 0.3% of the amount produced by zona fasciculata-reticularis cells.

Measurement of Prostaglandin-Stimulated Aldosterone Release

At confluence, each culture well was washed twice with 1 mL modified Ham’s F-12 medium containing 1 mg/mL BSA. The cells were incubated for 2 hours in this medium. It was then replaced with 1 mL of Ham’s F-12 containing 2 mg/mL BSA and 1.8 mmol/L calcium chloride, and prostanoids were added. The incubation was continued for 1 hour at 37°C. As a positive control for the functional integrity of the cells, angiotensin II was added routinely to one set of cells. All prostanoids were added in a volume of 10 μL, and an equal volume of the vehicle was added to the control cells. Prostanoids were diluted in incubation medium immediately before an experiment. In studies in which receptor antagonists or calcium channel blockers were used, the antagonist or its vehicle was added 10 minutes before the addition of the stimulus, and the incubation was continued for an additional 60 minutes. After incubation, the medium was removed, frozen, and stored until assayed. All experiments were performed on two to five different cell preparations. The data represent pooled results from multiple incubations from different cell preparations or summarized results from a representative experiment that was performed on two or three cell preparations.

Aldosterone was measured by direct radioimmunoassay, as previously described or by ELISA. Briefly, in the radioimmunoassay method, the sample was incubated with phosphate buffered saline containing sodium azide and polyvinylpyrrolidone. 3H-Aldosterone and sheep anti-aldosterone antibody were added in a total volume of 0.3 mL. All samples and unlabelled aldosterone standards were assayed in duplicate. After they were incubated overnight at 4°C, bound and free aldosterone were separated with dextran-coated charcoal. The bound counts were measured by liquid scintillation spectrometry. Results were expressed as picograms of aldosterone/mL. Aldosterone was measured by ELISA using a mouse anti-aldosterone monoclonal primary antibody and aldosterone–horseradish peroxidase conjugate provided by Dr C.E. Gomez-Sanchez (Truman VA Medical Center, Columbia, Mo) and a goat anti-mouse, Fc fragment–specific secondary antibody (Jackson Immunoresearch). The cross-reactivity of the primary antibody was as follows: aldosterone 100%, cortisol <0.0025%, corticosterone <0.0025%, DOC <0.0025%, progesterone <0.0025%, 18-OH DOC <0.0065%, and cortisone <0.0025%. Aldosterone was directly assayed by the addition of incubation media to 96-well ELISA plates precoated with secondary antibody. ELISA 96-well plates were precoated with the secondary antibody by incubating 300 μL of a 3.3 μg/mL solution of the goat anti-mouse IgG in 0.1 mol/L Na_2CO_3, pH 9.6, for 18 hours at 4°C. The plates were then washed three times with 300 μL/well of buffer containing 135 mmol/L NaCl, 20 mmol/L NaHPO_4, 0.01% thimerosal, and 0.2% Tween-80 (wash buffer) using a BioTek model EL402 automatic plate washer. Coated ELISA plates were stored in 10 mmol/L phosphate buffered saline containing 138 mmol/L NaCl and 2.7 mmol/L KCl at 4°C until used. The aldosterone–horseradish peroxidase conjugate and anti-aldosterone antibody were each diluted 1:6000 in the assay buffer containing 150 mmol/L NaCl, 100 mmol/L NaHPO_4, 0.1% Tween-80, 0.01% thimerosal, and 0.5% BSA. The assay buffer (250 μL) was added to 50 μL of the standard or sample in each well. The assay was then allowed to equilibrate overnight at 4°C. The plates were washed six times with 300 μL/well wash buffer with an automatic plate washer with a 1-minute agitation on an orbital shaker after the third wash. The assay was developed by the addition of 0.01% urea peroxide in 100 mmol/L citric acid and 40 mmol/L 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) as a color reagent. Aldosterone was quantified by colorimetric measurement using a Bio–Tek model EL309 automated plate reader with a 490 nm filter. Statistical analysis was performed using one-way ANOVA, followed by Student’s t test when differences were found to be significant. P < 0.05 was considered statistically significant.

Intracellular Calcium Measurements in Zona Glomerulosa Cells

Intracellular calcium was measured as previously described. Cells were cultured on coverslips for 1 to 5 days. Coverslips were washed in 10 mmol/L HEPES buffer (pH 7.4) containing 155 mmol/L sodium chloride, 5 mmol/L potassium chloride, 1.8 mmol/L calcium chloride, 1 mmol/L magnesium chloride, and 5.5 mmol/L glucose (buffer-I). Cells were equilibrated with 10 μmol/L fura-2 AM and 0.1% pluronic acid in buffer-I for 30 minutes at 24°C and then washed in buffer-I. A coverslip was then mounted on an open Sykes-Moore chamber placed on a microscope stage (Nikon) maintained at 37°C, and 0.5 mL buffer-I was added. A cluster of zona glomerulosa cells was chosen using a 40× objective. Using a dual excitation fluorimeter (Photon Technologies Inc), excitation light from two monochromators was alternated rapidly between wavelengths of 350 and 380 nm. Emission fluorescence was measured via computer synchronization by a photomultiplier tube through a 400-nm dichroic mirror and a 510-nm filter. After a stable baseline was achieved, angiotensin II, dibutyryl cAMP, or PGE_2 were added. Cells were treated with 5 μmol/L imonoycin in dimethyl sulfoxide followed by 100 mmol/L EGTA to measure maximum and minimum fura-2 fluorescence. After subtracting background fluorescence in preloaded cells, the 350/380 fluorescence ratio was determined, and intracellular calcium was calculated based on the method of Grynkiewicz et al.
Prostaglandins Studied and Their Selectivity for Prostanoid Receptors

<table>
<thead>
<tr>
<th>Receptor Specificity</th>
<th>Agonist or Antagonist</th>
<th>Manufacturer/Distributor</th>
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<tr>
<td>EP</td>
<td>PGE₂</td>
<td>Cayman Chemical, Ann Arbor, Mich</td>
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<tr>
<td>EP₁</td>
<td>17-phenyl trinor PGE₂</td>
<td>Cayman Chemical, Ann Arbor, Mich</td>
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<tr>
<td>EP₂</td>
<td>11-deoxy PGE₁</td>
<td>Schering, Germany</td>
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<tr>
<td>EP₃/EP₁</td>
<td>Sulprostone</td>
<td>Schering, Germany</td>
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<tr>
<td>EP₁</td>
<td>SC-19220</td>
<td>Wellcome, United Kingdom</td>
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Analysis of cAMP Generation

Twenty-four–well plate cultures of bovine zona glomerulosa cells were used. The Ham's F-12 culture medium was replaced by media containing penicillin (50 U/mL), streptomycin (50 µg/mL), fungizone (2.5 µg/mL), 1.4 µmol/L flurbiprofen, and 1.0 mmol/L isobutylmethylxanthine, a phosphodiesterase inhibitor. After a 15-minute preincubation at 37°C, the medium was replaced with 0.5 mL of EBSS buffer containing the above inhibitors, with or without prostanoid agonists, at the desired concentration. After an additional 15-minute incubation, the reaction was terminated by removal of the incubation buffer and the addition of 0.25 mL of 0.5 N NaOH. The cells were removed by transfer pipette to polycarbonate tubes and then vortexed. After 5 minutes, samples were neutralized with 0.25 mL of 0.5 N HCl and then centrifuged for 15 minutes at 3000g in an Eppendorf centrifuge. Aliquots (50 µL) of supernatant were assayed for cAMP by radioimmunoassay using an Amersham kit according to the manufacturer’s recommendations. The amount of cAMP formed was expressed as picomoles per well.

Materials and Compounds

The prostanoids studied and their selectivity for prostanoid receptors are shown in the Table.

Other Compounds

The anti-aldosterone serum was generously provided by the Pituitary Hormone Distribution Program of the NIH. The following reagents were used: a specially modified low-sodium Ham's F-12 medium, DME/F-12 medium, and flurbiprofen (Sigma Chemical Co); EBSS, horse serum, and antibiotic-antimycotic solutions (GIBCO); fetal bovine serum (Hyclone); collagenase type I (Worthington Biochemical Corp); dispase (Boehringer Mannheim Biochemicals); cAMP radioimmunoassay kits (Amersham); fura-2 AM (Molecular Probes); and 3H-cAMP (Amersham and New England Nuclear). All other reagents were purchased from Sigma Chemical Co.

Data Analysis and Collection

Data were analyzed using Sigma Plot (Jandel Scientific) to fit data by an iterative process to the four parameter logistic equation. This analysis produced a “best fit” to a sigmoidal curve. The analysis was performed using Student’s t test. A value of P<.05 was considered statistically significant.

Results

Prostaglandin-Stimulated Aldosterone Release

PGE₁ stimulated aldosterone release from cultured bovine zona glomerulosa cells in a concentration-related manner, with half-maximal stimulation occurring at approximately 300 nmol/L (Fig 1). Angiotensin II also stimulated aldosterone release in a concentration-related manner with half-maximal stimulation occurring at approximately 200 pmol/L (Fig 1). Although less potent than PGE₂, the EP₁ agonist 11-deoxy PGE₁ stimulated release with a half-maximal concentration of 2 µmol/L. The response to this analogue was approximately 70% of the aldosterone release due to PGE₂ (Fig 1). The EP₁-selective agonist 17-phenyl trinor PGE₁ (EP₂ selective agonist), produced a stimulation of aldosterone release at the 100 µmol/L concentration (Fig 1). Sulprostone, an EP₂/EP₃ selective agonist, failed to alter aldosterone release at any of the concentrations tested (Fig 1). The EP₁ antagonist SC-19220 did not alter the PGE₁-stimulated (100 nmol/L) aldosterone release at any of the concentrations tested (Fig 2). SC-19220 also had no effect on basal aldosterone release (data not shown). Dibutyryl cAMP (1 mmol/L) stimulated aldosterone release by 3.5-fold (Fig 3).

The calcium channel inhibitor diltiazem inhibited basal aldosterone release. In addition, the stimulation resulting from PGE₁ and dibutyryl cAMP was reduced to basal levels by treatment with diltiazem (P<.01) (Fig 3).

![Graph showing aldosterone release](image-url)
Stimulation of Intracellular Calcium by Angiotensin II, Dibutyryl cAMP, and PGE₂

Using fura-2–loaded ZG cells, both dibutyryl cAMP (2 mmol/L) and PGE₂ (2 μmol/L) increased intracellular calcium (Fig 4A and 4B, respectively). Angiotensin II (2 μmol/L) also increased intracellular calcium (Fig 4C). The effect of dibutyryl cAMP was more prolonged than the effect of PGE₂ or angiotensin II. When the results from a number of experiments were summarized (Fig 4D), dibutyryl cAMP increased intracellular calcium by 3-fold, PGE₂ increased intracellular calcium by 5.5-fold and angiotensin II increased intracellular calcium by 6.2-fold (P<.01).

Prostaglandin Stimulation in cAMP Formation

Fig 5A indicates the time course of intracellular, extracellular, and total cAMP production after stimulation by PGE₂ (1 μmol/L). There was a rapid increase in the production of cAMP during the first 10 minutes after exposure to PGE₂ that then slowed during the subsequent 35 minutes. Intracellular cAMP did not change during this 35-minute interval; however, extracellular cAMP continued to rise slowly. The rate of production was rapid and exceeded half-maximal levels within 5 minutes of exposure to PGE₂. Basal production of cAMP in the absence of PGE₂ stimulation was 0.7 pmole per well or 17.5 pmole per mg protein with a 10% to 15% variation.

We chose 15 minutes as the incubation interval for the remainder of the experiments because intracellular cAMP concentrations are maximal and constant at this interval. Fig 5B illustrates the concentration–response curve obtained for PGE₂ (10 nmol/L to 100 μmol/L). The ED₅₀ for PGE₂ stimulation of cAMP was 10 μmol/L. Although the ED₅₀ value was in the micromolar range, changes in cAMP levels were quantifiable with nanomolar amounts of PGE₂. As much as 700 to 900 pmole of cAMP per mg protein was measured in response to the 100 μmol/L PGE₂, equivalent to more than a 100-fold increase over basal values. PGE₂ and the EP₂ agonist 11-deoxy PGE₂ caused a significant increase in the amount of cAMP (Fig 6). The EP₁- and EP₃/EP₁-selective agonists did not stimulate cAMP production.

Discussion

We and others have previously reported that PGE₂ stimulates the release of aldosterone from acutely prepared rat adrenal glomerulosa cells.¹⁴–¹⁸ PGE₂ increases aldosterone release by stimulating the early step in aldosterone biosynthesis, the conversion of cholesterol to pregnenolone.¹⁵ The concentrations of PGE₂ required for aldosterone release varied widely between studies, from nanomolar to micromolar amounts. The reasons for the differences in responsiveness are not clear, but they may be due to the methods used to prepare the zona glomerulosa cells. For example, Dazord and coworkers found that trypsin treatment inhibits binding of ³H-PGE₂ to adrenal membranes. Since trypsin was used in some instances to dissociate the cells, its use may explain the various responses to PGE₂. In the present study, bovine zona glomerulosa cells were maintained in primary culture for 5 to 7 days to avoid some of the variability that may occur with acutely isolated cells. PGE₂ was a moderately potent agonist in stimulating the release of aldosterone from these cultured zona glomerulosa cells. The half-maximal concentration for PGE₂ stimulation of aldoste-
PGE₂ elicited a maximal release of aldosterone that was approximately 75% of the maximal aldosterone released in response to angiotensin II. The inability of PGE₂ to stimulate aldosterone release to the same extent as angiotensin II is probably because they act through different mechanisms. As indicated in these studies, PGE₂ acts through an increase in cAMP and an increase in intracellular calcium. Angiotensin II activates phospholipase C, increasing DAG and IP₃. This latter compound will also increase intracellular calcium. The remaining aldosterone release may be attributed to DAG and calcium activation of protein kinase C. Previous studies have indicated that protein kinase C activation is critical for the sustained phase of aldosterone release. The ability of PGE₂ to stimulate the release of aldosterone in physiologically relevant concentrations supports a potential role for this prostanoid in the regulation of aldosterone release.

After establishing that PGE₂ could release aldosterone, we determined which subtype of the EP receptor mediated this action. We used prostanoid agonists selective for three of the known subtypes of the EP receptor to examine their ability to stimulate either second messenger production or aldosterone release. The EP₁ receptor second messenger is IP₃/DAG, and its activation is accompanied by calcium mobilization. Activation of an EP₂ or EP₃ receptor results in stimulation and inhibition, respectively, of adenylyl cyclase activity. This is reflected by an increase or decrease in intracellular cAMP concentration. It is also known that ACTH-stimulated aldosterone release is accompanied by the accumulation of cAMP, whereas angiotensin II stimulation is accompanied by calcium mobilization.

Only the EP₂ agonist 11-deoxy PGE₁ stimulated the release of aldosterone, whereas the EP₃ agonist sulprostone was without effect. This suggested that the second messenger cAMP was involved in the release of aldosterone. The EP₁ agonist 17-phenyl trinor PGE₂ elicited an aldosterone release; however, this occurred only at high micromolar concentrations. Therefore, we used the selective EP₁ antagonist SC-19220 to rule out the presence of a role for the EP₁ receptor. The EP₁ antagonist had no effect on PGE₂ stimulation of aldosterone, indicating that PGE₂ was not acting at an EP₁ receptor. The lack of activity of sulprostone and the elimination of a role for an EP₁ receptor pointed to the presence of an EP₂ receptor that mediated the PGE₂-induced release of aldosterone in the zona glomerulosa cells.

PGE₂ also stimulated the accumulation of cAMP in a concentration-related manner. Similar results have been reported by others. Since EP₂ receptor activation is associated with adenylyl cyclase activation and cAMP formation, these findings support our conclusion that the effect of PGE₂ is mediated by an EP₂ receptor. The half-maximal response for cAMP formation occurred at

Figure 5. Time course of cAMP production by cultured zona glomerulosa cells. Panel A indicates the time course of intracellular, extracellular, and total cAMP production after stimulation by PGE₂ (1 μmol/L). Basal production of cAMP in the absence of PGE₂ stimulation was 0.7 pmole per well or 17.5 pmole per mg protein with a 10% to 15% variation. Panel B illustrates the concentration-response curve obtained during a 15-minute exposure to PGE₂ (10 nmol/L to 100 μmol/L). As much as 700 to 900 pmole of cAMP per mg protein was measured in response to the 100 μmol/L PGE₂. Each value represents the mean±SEM for n=4.

Figure 6. Effect of prostaglandin EP receptor selective agonists on cAMP accumulation in cultured zona glomerulosa cells. cAMP was stimulated by a 15-minute exposure to agonists selective for each of the EP receptor subtypes. Each agonist was evaluated at a concentration of 10 μmol/L. The results are expressed as a pmole of cAMP/well. Each value represents the mean±SEM for n=4.

PGE₂ release was approximately 300 nmol/L. PGE₂ elicited a maximal release of aldosterone that was approximately 75% of the maximal aldosterone released in response to angiotensin II.
10 μmol/L, whereas the half-maximal stimulation of aldosterone release occurred at 300 nmol/L. The difference between these half-maximal responses is not unusual and has been reported for most tissues containing prostanoid receptors. It suggests that a small change in cAMP production is sufficient for the physiological response. The time course of cAMP release was rapid, achieving a maximum response within 10 minutes of agonist stimulation. The EP3 agonist was able to stimulate cAMP and aldosterone release, and its effects were approximately 70% of the maximal stimulation by PGE2. It is likely that 11-deoxy PGE1 does not fully activate the EP3 receptor subtype, as evidenced by its inability to achieve the same maximum stimulus as PGE2. This incomplete activation results in decreased cAMP generation and incomplete stimulation of aldosterone release.

Angiotensin II, PGE2, and dibutyl cAMP all increased intracellular calcium in fura-2-loaded zona glomerulosa cells. This suggests that CAMP and calcium both mediate the effects of PGE2, and that the increase in calcium may be secondary to the increase in cAMP. The calcium channel blocker diltiazem inhibited PGE2 and cAMP stimulation of aldosterone release. Therefore, the increase in cAMP alone is not sufficient to cause aldosterone release, but rather the increase in cAMP must be coupled to an increase in calcium. An analogous series of events has been proposed for ACTH. ACTH increases the accumulation of CAMP and release of aldosterone in zona glomerulosa cells. The release of aldosterone correlated with the increase in CAMP formation. A reduction in extracellular calcium decreased the steroidogenic response to ACTH and to 8-bromo-cAMP. Similarly verapamil inhibited ACTH-induced steroidogenesis. Some investigators have observed an increase in intracellular calcium with ACTH, whereas others have not. These findings indicate that cAMP and calcium both also mediate the steroidogenic effect of ACTH.

In summary, we conclude that PGE2-stimulated aldosterone release from bovine zona glomerulosa cells is mediated by an EP3 receptor subtype. It appears that PGE3, like ACTH, utilizes both cAMP and calcium as second messengers in the regulation of aldosterone release.

Acknowledgments

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References


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