Mechanism of Action of Aldosterone Release by Prostaglandin E in Isolated Rat Adrenal Glomerulosa Cells

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Abstract

The effect of prostaglandin E (PGE) on aldosterone release and the mechanism of action of PGE in mediating the release of aldosterone were studied using isolated rat glomerulosa cells. PGE₁ stimulated aldosterone release in a dose-dependent fashion at concentrations between 10⁻⁸ and 10⁻⁶ M and caused approximately a two-fold increase over the basal aldosterone level at 10⁻⁶ M. A significant and dose-dependent increase in cAMP production was also produced by PGE₁ at concentrations greater than 10⁻⁸ M. Aldosterone release induced by 10⁻⁷ M or 10⁻⁶ M PGE₂ was significantly reduced by a competitive receptor blocking PG-antagonist, SC 19220 (10⁻⁷ M), but not affected by (Sar¹, Ileu⁸)-angiotensin-II (A-II), a competitive inhibitor of A-II. PGE-stimulated aldosterone release was almost completely abolished by depleting the extracellular Ca²⁺ by EGTA, or by verapamil, a Ca²⁺-channel blocker or W-7, a calmodulin inhibitor.

These findings suggest that PGE stimulates aldosterone release through the membrane receptor binding and activation of adenylate cyclase and that Ca²⁺-calmodulin system plays an essential role in mediating the steroidogenic action of PGE in the adrenal glomerulosa cells. However, the physiological significance of PGE in the regulation of aldosterone secretion remains to be elucidated.

Earlier reports have shown that prostaglandin E (PGE) can directly stimulate adrenal glomerulosa cells to secrete aldosterone in various species (Saruta and Kaplan, 1972; Honn and Chavin, 1976; Enyedi, *et al.*, 1981). However, some investigators failed to confirm this direct stimulating effect of PG

on the release of adrenal steroids (Blair-West, *et al.*, 1971; Matsuoka, *et al.*, 1980). Thus, the currently available data on the direct effect of PGE on aldosterone release are still conflicting. Furthermore, the mechanism of action of PGE in stimulating aldosterone release has not yet been fully elucidated, although a few papers describe a concomitant increase in aldosterone and cyclic adenosine 3', 5'-monophosphate (cAMP) (Saruta and Kaplan, 1972) and the significance of the Ca²⁺-calmodulin system (Balla *et al.*, 1982a; Balla *et al.*, 1982b) in this process. In this study, a stimulative effect of PGE on aldosterone release was confirmed using

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isolated rat glomerulosa cells. In order to investigate the mechanism of action of PGE in stimulating aldosterone release, the significance of the receptor-adenylate cyclase system, extracellular Ca^{2+} and calmodulin in mediating steroidogenic action of PGE, were also evaluated, since all of these are known to be essential to the secretion of many of hormones.

Material and Methods

Synthetic (Asp¹, Ileu⁵)-angiotensin II (A-II) was obtained from Ciba Geigy Corp., Tokyo, Japan and (Sar¹, Ileu⁵)-A-II from the Peptide Institute, Inc., Osaka, Japan. PGE₁ and E₂ were kindly supplied by Ono Yakuhin Co., Ltd., Osaka, Japan, verapamil by Eisai Co., Ltd., Tokyo, Japan and SC-19220 by Searle Research and Development, IL, USA. N-(6-Amino-hexyl)-5-chloro-1-naphthalenesulfonamide (W-7) was purchased from Seikagaku Kogyo Co., Ltd., Tokyo, Japan. Ethyleneglycol-bis (β -amino ethyl ether) NN'-tetraacetic acid (EGTA), Hepes and bovine serum albumin (BSA, Cohn fraction V) were obtained from Sigma Chemical Corp., St. Louis, MO, USA and collagenase (type II) and deoxyribonuclease (1,400–1,800 U/mg) from Cooper Biochemical Corp., NJ, USA. Medium 199 was obtained from Gibco Laboratories, NY, USA.

The glomerulosa cell suspension was prepared using a modification of the method described by Haning *et al.* (1970) as follows. Male Sprague-Dawley rats (200–250 g) were purchased from the Charles River Laboratories, Tokyo, Japan and maintained on a normal sodium intake with water ad libitum. Rats were killed between 10 and 11 a.m. by decapitation under sodium pentobarbital anesthesia. The adrenal glands were removed, freed from fat and adherent tissue and divided into capsular and decapsular portions. The capsular portions which contain mainly glomerulosa cells were then minced into small pieces with scissors in 25 mM Hepes buffer, pH 7.4, containing 1 mg/ml glucose and washed three times with 10 ml Hepes buffer in a 10 ml polystyrene tube. The washed pieces of the capsular tissue were incubated in 10 ml of Hepes buffer containing 3.0% BSA, 0.3% collagenase and 0.2 mg/ml DNase (Hepes-collagenase-DNase) under constant shaking at 37°C for 20 min in a 50

ml glass flask. At the end of the incubation period, the tissues were transferred to another 50 ml glass flask containing 10 ml of Hepes-collagenase-DNase solution for dispersion. Dissociation of the cells was accomplished by pipetting up and expelling the tissue and solution in the flask 15 times at 10 min intervals for 20 min with a 5 ml plastic serological pipet and rubber suction bulb. The dispersed cells suspensions were centrifuged at 100 g for 7.5 min at 4°C. The supernatant was removed with a 5 ml pipet and the cell pellets were then filtered through 100 mesh stainless steel gauzes to remove undigested coarse materials into a 10 ml polystyrene tube. The cells were washed three times with 10 ml medium 199 containing 0.2% BSA, and diluted appropriately with the medium in a 50 ml conical polypropylen tube (Falcon, #2070).

The cells (5×10^4 cells) were incubated in 0.5 ml aliquots of medium 199 containing 0.2% BSA with or without various agents in a polystyrene tube in triplicate at 37°C for 2 hours in an atmosphere of 95% O₂ and 5% CO₂. After incubation, cell-free aliquots of medium were stored at –20°C for later determination of aldosterone and cAMP.

The concentration of aldosterone in the culture medium was determined in duplicate by direct radioimmunoassay (RIA) with the specific antiserum to aldosterone raised in rabbit which was a kind gift from Dr. Kanbegawa, Department of Gynecology and Obstetrics, Teikyo University School of Medicine, Tokyo, Japan. cAMP was measured by RIA using a commercial kit obtained from Yamasa Shoyu Co., Ltd., Chiba, Japan. Results are presented as the mean of triplicate determinations $\pm$ SEM unless otherwise indicated. Statistical analysis was performed using Student's unpaired *t*-test.

Results

The effect of PGE₁ on aldosterone release in the present experimental system is shown in Fig. 1. PGE₁ significantly stimulated aldosterone release in a dose-dependent fashion at concentrations greater than 10^{-8} M and the aldosterone concentration stimulated by 10^{-6} M PGE₁ was 5.90 ± 0.16 ng/ 10^5 cells/ml which was approximately twice the control value (2.54 ± 0.18 ng/ 10^5 cells/ml). cAMP

Fig. 1. Effect of PGE₁ on aldosterone and cAMP concentration in the incubation medium. The asterisks designate those points that are significantly different from the corresponding control value, and each point and bracket represent the mean and SEM, respectively as in the following figures.

Fig. 2. Effect of SC 19220, a competitive PG-receptor blocker, on PGE₂-stimulated aldosterone release. Cells were incubated with either PGE₂ (10⁻⁹~10⁻⁶M) alone or in the presence of 10⁻⁷M SC 19220.

production was also significantly increased by PGE₁ in a dose-dependent fashion at the same concentration as stimulated aldosterone release (Fig. 1).

In order to clarify whether or not PGE exerts a steroidogenic action through the cell membrane receptor, the glomerulosa cells were co-incubated with PGE (10⁻⁷ M) and various concentrations of a competitive plasma membrane receptor blocker for PG, SC 19220 (Kennedy *et al.*, 1983). Aldosterone release stimulated by PGE₂ was

significantly suppressed in the presence of 10⁻⁷ to 10⁻⁶ M SC 19220. However, a slight rise in the aldosterone level was observed when the concentration of PGE₂ was between 10⁻⁹ and 10⁻⁸ M, which probably indicates the agonistic effect of this compound on the action of PGE₂ (Fig. 2).

The cells were incubated with constant concentrations of PGE₁ (10⁻⁶ M), PGE₂ (10⁻⁶ M) or A-II (10⁻⁷ M) and various concentrations (10⁻⁸ M to 10⁻⁵ M) of a competitive inhibitor of A-II, (Sar¹, Ileu⁸)-A-II,

Fig. 3. Effects of (Sar¹, Ileu⁸)-angiotensin-II on aldosterone release stimulated by PGE₁, PGE₂ or angiotensin-II. Concentrations of (Sar¹, Ileu⁸)-angiotensin II are plotted on the abscissa.

Fig. 4. Effect of various concentrations of EGTA on PGE-stimulated aldosterone release.

in order to find whether or not PGE shares the receptor binding sites with those of A-II. Aldosterone release stimulated by 10⁻⁷ M A-II was significantly suppressed by this competitive inhibitor. However, PGE-stimulated aldosterone secretion was not modified by this inhibitor (Fig. 3).

Aldosterone release stimulated by 10⁻⁶ M PGE₁ or E₂ was significantly decreased by EGTA, a Ca²⁺-chelator, added to the incubation medium (Fig. 4). The addition of 2x10⁻³ M EGTA to the medium sup-

pressed PGE (10⁻⁶ M)-stimulated aldosterone release to approximately 17% of the control value (Fig. 4). This suppressive effect of EGTA on PGE-stimulated aldosterone release was completely reversed by adding Ca²⁺ to the medium (Fig. 5), indicating that the release of aldosterone by PGE is dependent on the extracellular concentration of Ca²⁺ and that inhibition of PGE-stimulated aldosterone release by EGTA is due to the decrease in extracellular Ca²⁺, and EGTA per se has no inhibitory effect on aldosterone secretion.

Fig. 5. Effect of various concentrations of Ca^{2+} in the medium of PGE-stimulated aldosterone release. Various amounts of CaCl_2 was added to the incubation medium which contained 10^{-6} M PGE and 2×10^{-3} EGTA, and the concentration of Ca^{2+} was measured and plotted on the abscissa (0, 5, 160, 945, 1045 μM).

Fig. 6. Effect of verapamil, a Ca^{2+} -channel blocker, on PGE-stimulated aldosterone release.

Verapamil which is known to be a Ca^{2+} channel blocker (Singh *et al.*, 1978), significantly antagonized the response of glomerulosa cells to PGE_1 or PGE_2 at 10^{-5} M as shown in Fig. 6. In order to examine the significance of calmodulin in mediating the steroidogenic action of PGE, a selective calmodulin inhibitor, W-7 (Hidaka *et al.*, 1981), was added to the medium in the presence of 10^{-6} M PGE_1 , and aldosterone and cAMP in the medium were measured after the incubation. Significant and dose-

dependent suppression of the aldosterone level was observed with W-7 at a concentration greater than 10^{-5} M (Fig. 7) and W-7 at 3×10^{-5} M caused almost complete inhibition (approximately 7% of the control value) of PGE_1 -stimulated aldosterone release.

Discussion

Our present data clearly show that PGE

Fig. 7. Effect of W-7, a selective calmodulin inhibitor on PGE₁-stimulated aldosterone release.

stimulates aldosterone release by directly acting on the glomerulosa cells, which are associated with cAMP production (Fig. 1). The following is a possible explanation for the discrepancy between the present results and those of others (Matsuoka *et al.*, 1980) who have failed to demonstrate the direct stimulating effect of PGE on the adrenal cortex in secreting aldosterone, in spite of using similar *in vitro* methods. PG-receptor on the cell membrane could be, to some extent, destroyed or modified depending upon the enzymes and mechanical force employed to isolate the glomerulosa cells, as has been suggested (Matsuoka *et al.*, 1980; Enyedi *et al.*, 1981), or may be sensitive to trypsin inhibitors (Pedersen and Brownie, 1983) used often to neutralize the trypsin activity at the end of dispersion of the cells. In such cases, the cells may become less responsive to the ligands.

The present study suggests that the stimulating action of PGE on aldosterone release in the glomerulosa cells is mediated through the PG-receptor, which is not related to A-II receptor, and adenylate cyclase system for the following reasons. Firstly, a parallel increase in cAMP and aldosterone level caused by PGE was observed (Fig. 1). Secondly, the stimulating effect of PGE is

significantly eliminated by SC 19220 (Kennedy *et al.*, 1983), a competitive plasma membrane receptor blocker for PG (Fig. 2), but not by (Sar¹, Ileu⁸)-A-II, a competitive inhibitor of A-II (Fig. 3). These findings are in good agreement with those of Saruta *et al.* (1972) who have found a concomitant increase in aldosterone and cAMP levels caused by PGE in bovine adrenal slice and Dazord *et al.* (1974) and Shima *et al.* (1980) who have shown, using ovine adrenal preparation, that PGE₁ binds to the plasma membrane receptor and stimulates cAMP production.

Calmodulin is an intracellular Ca²⁺-receptor protein which regulates many enzymes and is found in a number of tissues including the adrenal gland (Harper *et al.*, 1980; Cheung, 1980; Weiss *et al.*, 1980). Ca²⁺ and calmodulin play important roles in mediating the action of a variety of hormones and biologically active substances (Harper *et al.*, 1980). It is well established that the steroidogenesis by ACTH (Farese, 1971; Leier and Jungmann, 1973; Haksar and Péron, 1973; Fakunding *et al.*, 1979; Balla *et al.*, 1982a), A-II (Chiu and Freer, 1979; Fakunding *et al.*, 1979; Capponi *et al.*, 1984) and K⁺ (Fakunding and Catt, 1980; Capponi *et al.*, 1984) are highly dependent

upon the Ca^{2+} -calmodulin system. A significant role of Ca^{2+} and calmodulin in mediating the steroidogenic action of PGE has been reported, but only in a few papers (Balla *et al.*, 1982a; Balla *et al.*, 1982b). In the present study, it is shown that PGE-stimulated aldosterone is suppressed by 1) depletion of extracellular Ca^{2+} by EGTA (Fig. 4), 2) verapamil, a Ca^{2+} -channel blocker (Singh *et al.*, 1978), (Fig. 6) or 3) W-7, a selective calmodulin-inhibitor (Hidaka *et al.*, 1981) (Fig. 7). These findings indicate that both Ca^{2+} -influx and the function of Ca^{2+} -calmodulin system within the cytoplasm are very important in mediating the action of PGE on aldosterone release. Thus, it is concluded that the mechanism of action of PGE in stimulating aldosterone secretion in the adrenal cortex is similar to that of ACTH in that both cAMP and Ca^{2+} -calmodulin systems play essential roles in its steroidogenic action.

PG's are very rapidly metabolized into inactive forms mainly in the lung (Piper *et al.*, 1970; Samuelson *et al.*, 1971), and the concentration of PGE in stimulating aldosterone release observed in our experiments appears to be higher than that normally encountered in the circulation (Hertelendy *et al.*, 1973; Jaffe and Behrman, 1979). Thus it may be unlikely, at least in normal conditions, that PGE produced in tissue remote from the adrenal gland acts, via the circulation like classical hormones, on the adrenal cortex to stimulate the steroidogenesis. However, in certain pathological conditions, production of PGE may be increased (Jaffe and Behrman, 1979) to stimulate aldosterone secretion. It is also quite possible that PGE produced within the adrenal gland stimulates or modulates steroidogenesis as tissue hormone, although this possibility was not examined in this study. Further studies are required to establish the exact physiological significance of PGE in the regulation of aldosterone secretion.

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