ROLE OF PROSTAGLANDINS IN THE CONTROL OF THE FUNCTION OF ADRENAL GLOMERULOSA CELLS

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(Received 24 February 1981)

SUMMARY

The role of prostaglandins in the control of aldosterone production was studied in isolated rat glomerulosa cells. Exogenous prostaglandin E₂ in concentrations above 10⁻⁹ mol/l increased the production rate of aldosterone; this effect was attenuated by the competitive antagonist, 7-oxa-13-prostynoic acid. Prostaglandin F₂α (10⁻⁹–10⁻⁵ mol/l) failed to influence the production rate of aldosterone. The aldosterone-stimulating effect of the prostaglandin precursor, arachidonic acid (5×10⁻⁴ mol/l), could not be blocked by inhibitors of prostaglandin synthesis. Basal production rate of aldosterone was not significantly influenced by non-steroidal anti-inflammatory drugs. Glomerulosa cells were stimulated by angiotensin II; this effect was not potentiated by arachidonic acid and was reduced only slightly by indomethacin. The cells were also stimulated by corticotrophin and potassium ions. The effect of these substances was not potentiated by arachidonic acid and was not inhibited by non-steroidal anti-inflammatory drugs. These results do not confirm the presumption that intra-adrenal prostaglandins play an essential role in the control of aldosterone secretion. Some effects of arachidonic acid and its antagonist, eicosatetraynoic acid, on aldosterone production are considered to be independent of changes in prostaglandin synthesis.

INTRODUCTION

The effect of prostaglandins on steroid secretion has been under examination for several years, but the role of intra-adrenal prostaglandins in the control of aldosterone secretion is far from being established. The metabolism of prostaglandins in the adrenal zona glomerulosa has not been systematically studied, until now the synthesis of only two primary prostaglandins, prostaglandin (PG) E₂ and PGF₂α, having been demonstrated (Campbell, Gomez-Sanchez, Adams, Schmitz & Itskovitz, 1979; Matsuoka, Tan & Mulrow, 1980; Miller, Douglas & Dunn, 1980). Prostaglandin E₂ has been found to increase the production rate of aldosterone by incubated rat (Spät, Sarkadi, Intódy, Körner & Szántó, 1971; Spät & Józan, 1975; Campbell, Gomez-Sanchez & Adams, 1980), bovine (Saruta & Kaplan, 1972) and human (Honn & Chavin, 1976) adrenal tissue or cells but was without effect when infused into the adrenal artery of the sheep (Blair-West, Coghlan, Denton, Funder, Scoggins & Wright, 1971). Reports on the effect of PGF₂α are conflicting (Saruta & Kaplan, 1972; Honn & Chavin, 1976; Spät, Antoni, Balla, Bonta & Siklós, 1977). Prostaglandin A₂ exerts a stimulatory action on incubated rat glomerulosa tissue (Spät & Józan, 1975) and PGA₁ has a dose-dependent effect on incubated diced human adrenal (Honn & Chavin, 1977). The physiological relevance of these data, however, is rather doubtful as circulating or intra-adrenal PGA has not been demonstrated. The endoperoxide PGH₂ failed to alter

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aldosterone production by rat glomerulosa cells (Campbell et al. 1980) and data are not available concerning the effects of prostacyclin on the synthesis of aldosterone.

Altered production rate of a prostaglandin in response to angiotensin II, potassium ion or corticotrophin (ACTH), the known physiological stimulators of aldosterone secretion, would accord with the presumption of the physiological role of prostaglandins in the control of the function of glomerulosa cells. No change in the release of PGE$_2$ or PGF$_{2\alpha}$ by isolated glomerulosa cells in response to angiotensin II and III (Campbell et al. 1980; Miller et al. 1980) or potassium ion (Miller et al. 1980) has been demonstrated. Increased PGE$_2$ content in glomerulosa cells after stimulation with ACTH has been found in one experiment by Matsuoka et al. (1980), but no change was observed by Miller et al. (1980).

Another approach to assess the role of intracellular prostaglandins is to monitor the interaction between inhibitors of prostaglandin synthesis and the known stimulators of aldosterone secretion. Since the few published data (detailed in the Discussion) are also conflicting, we have systematically examined the effect of PGE$_2$, PGF$_{2\alpha}$, the prostaglandin precursor, arachidonic acid, and that of several inhibitors of prostaglandin synthesis on basal and stimulated aldosterone production by isolated rat glomerulosa cells. The results presented in this paper do not confirm the physiological significance of intra-adrenal prostaglandins in the control of aldosterone secretion.

**MATERIALS AND METHODS**

**Materials**

Deoxyribonuclease (DNase, from bovine pancreas, 450,000 Kunitz units/g) was purchased from Serva (Heidelberg, Germany); soybean trypsin inhibitor from Reanal (Budapest, Hungary); Medium 199 from Wellcome (Beckenham, Kent); human serum albumin (fraction V; HSA) from Humán (Budapest, Hungary); angiotensin II ([Asp$^1$, Val$^5$]-angiotensin II-$\beta$-amide, Hypertensin-Ciba) from CIBA-GEIGY (Basle, Switzerland); arachidonic acid from Sigma (St Louis, Missouri, U.S.A.) and PGF$_{2\alpha}$ (Enzaprostan F) from ChinoJ (Budapest, Hungary).

Antiserum to aldosterone was generously provided by the NIAMDD (Bethesda, Maryland, U.S.A.); 1–24 ACTH (Synacthen) from CIBA-GEIGY; indomethacin and niflumic acid from ChinoJ; carprofen (RO 2057) and 5,8,11,14-eicosatetraynoic acid (RO 3–1428; ETY) from Hoffmann–La Roche (Nutley, New Jersey, U.S.A.) and meclofenamic acid from Parke–Davis (Ann Arbor, Michigan, U.S.A.). Crude collagenase (type I, produced by Worthington, Freehold, New Jersey, U.S.A.) was a gift from Dr E. Bojesen (University of Copenhagen, Copenhagen, Denmark); 7-oxa-13-prostynoic acid from Dr J. Fried, Chicago, Illinois, U.S.A. and PGE$_2$ from Dr J. E. Pike (Upjohn Co., Kalamazoo, Michigan, U.S.A.).

The activity of indomethacin, carprofen, ETY and meclofenamic acid ($10^{-4}$ mol/l) was tested on incubated renal papillary slices. Each inhibitor reduced the immunoreactive PGE production below 10% and immunoractive PGF$_{2\alpha}$ production below 5% of the control value. The purity of ETY and 7-oxa-13-prostynoic acid was checked by mass spectrometry.

Materials used in the steroid analysis have been described previously (Spät, Síklós, Antoni, Nagy & Szirányi, 1977).

**Preparation and incubation of isolated glomerulosa cells**

The glomerulosa cell suspension was prepared using a modification of the method described by Fredlund, Saltman & Catt (1975). Briefly, male Sprague–Dawley (CFY) rats were maintained on a normal semi-synthetic diet (LATI, Gödöllő, Hungary) for at least 1 week before decapitation. The adrenal glands were removed and kept in potassium-free Krebs–Ringer bicarbonate–glucose (KRBG) solution until cleaned of fat and separated into capsular (mainly glomerulosa) and decapsulated portions by the method of Giroud,
Stachenko & Venning (1956). The capsular portions were cut into two to three pieces and repeatedly aspirated into a plastic pipette. The remaining tissue was digested for 25 min in potassium-free KRBG solution containing HSA (2 g/l) and collagenase (2 g/l) at 37 °C under an atmosphere of 95% O₂ and 5% CO₂. The solution was removed and the cells were dispersed by repeated pipetting in a potassium-free KRBG solution containing HSA (2 g/l), DNAse (0.1 g/l) and trypsin inhibitor (0.2 g/l) (dispersing solution).

The supernatant solution containing the cells was transferred into a centrifuge tube. The sedimented tissue was digested and dispersed again as above. The supernatant fractions were pooled and centrifuged for 10 min at 100 g at 4 °C. The cells were resuspended and centrifuged again in the dispersing solution and finally resuspended in a mixture of Medium 199 and potassium-free KRBG (2:1, v/v) containing HSA (2 g/l). The yield was approximately 10⁵ cells/rat. The cell suspension (0.5 ml) was mixed with KRBG containing 2 g HSA/l (0.5 ml) in Teflon vials. Potassium concentration was 3.6 mmol/l unless indicated otherwise. Angiotensin II and ACTH were added in 10 μl 0.15 mol saline/l (pH 4). Arachidonic acid, PGE₂, ETY, 7-oxa-13-prostynoic acid, niflumic acid, carprofen and meclofenamic acid were dissolved and added to the samples in ethanol. The final concentration of ethanol in all the tubes, including the control ones, was 0.5% (v/v). The cells were incubated at 37 °C for 90 min under an atmosphere of 95% O₂ and 5% CO₂. Basal aldosterone production rate was 3.18 ± 0.41 (s.e.m.) pmol/adrenal per 90 min in the 43 experimental series included in the present paper.

Steroid analysis

Aldosterone was estimated by means of radioimmunoassay as previously described (Enyedi & Spät, 1981), and corticosterone was estimated by competitive protein binding assay as detailed by Spät, Siklós et al. (1977).

Statistical analysis

With regard to the considerable variation in the production rate of hormones between separate experiments, the values are expressed by dividing the production rate of the stimulated sample by that of the matched, non-stimulated control sample. Means ± s.e.m. or, in some cases, individual values are given. The number of observations indicates the means of duplicate incubations from n separate experiments. The significance of the effect of the examined substances was estimated by means of three-way analysis of variance (Brownlee, 1961) or paired-sample t-test.

RESULTS

Effect of PGE₂, PGF₂α, arachidonic acid and prostaglandin inhibitors on basal aldosterone production

Exogenous PGE₂ in concentrations above 10⁻⁹ mol/l stimulated aldosterone production (Fig. 1). 7-Oxa-13-prostynoic acid (10⁻⁴ mol/l), the competitive antagonist of PGE₂, reduced the effect of PGE₂. The antagonist inhibited the response to 10⁻⁷ mol PGE₂/l by 64.5 ± 2.8 (s.e.m.) % (n = 3 duplicates) and that to 3 × 10⁻⁷ mol PGE₂/l by 34.3 or 26.0% (n = 2 duplicates). The aldosterone production rate in the presence of the highest concentration (10⁻⁶ mol/l) of PGE₂ examined was not significantly influenced by the antagonist (n = 3 duplicates). Prostaglandin F₂α (10⁻⁹–10⁻⁵ mol/l) failed to influence the production rate of aldosterone (Fig. 1).

Arachidonic acid at a concentration of 5 × 10⁻⁶ and 10⁻⁴ mol/l did not affect basal aldosterone output (n = 3 duplicates) while 5 × 10⁻⁴ mol arachidonic acid/l induced a 3.29 ± 0.38-fold increase in the production rate of the hormone (n = 7 duplicates). The non-steroidal anti-inflammatory drugs, indomethacin (3 × 10⁻⁵ mol/l) and niflumic acid (10⁻⁴ mol/l), failed to abolish the stimulatory effect of arachidonic acid.

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The basal production rate of aldosterone was not significantly modified by several non-steroidal anti-inflammatory drugs (indomethacin: $3 \times 10^{-5}$ mol/l, $n = 21$ duplicates; niflumic acid, carprofen and meclofenamic acid: each at $10^{-4}$ mol/l, $n = 2$ duplicates) or by 7-oxa-13-prostynoic acid ($10^{-4}$ mol/l, $n = 2$ duplicates). In contrast, ETY ($10^{-4}$ mol/l), the competitive antagonist of arachidonic acid, enhanced aldosterone production rate by $45.7 \pm 13.6\%$ ($P < 0.01$, $n = 19$ duplicates).

The effect of arachidonic acid on the stimulated aldosterone production

For a better assessment of the role of intra-adrenal prostaglandins in the control of aldosterone synthesis we tested whether the prostaglandin precursor, arachidonic acid,
potentiated the response of glomerulosa cells to its physiological stimuli. Arachidonic acid in a concentration of $5 \times 10^{-6}$ mol/l, which has no effect per se on the production of aldosterone, also failed to modify the response to angiotensin II (1 and 25 nmol/l), ACTH ($10^{-8}$ and $3 \times 10^{-7}$ mol/l) or potassium (5.7 and 8.2; as compared with 3.6 mmol/l) ($n = 3$ duplicates). The effect of $5 \times 10^{-4}$ mol arachidonic acid/l (Fig. 2) was additive with that of potassium, while angiotensin II- or ACTH-stimulated aldosterone production rates were even lower in the presence of arachidonic acid than in its absence.

**Effect of inhibitors of prostaglandin synthesis on the function of the stimulated glomerulosa cells**

The effect of angiotensin II on aldosterone production rate is shown in Fig. 3a. The minimal effective concentration was $2.5 \times 10^{-10}$ mol/l ($P < 0.001$, $n = 7$), and the maximal response was attained by $2.5 \times 10^{-8}$ mol/l. Indomethacin reduced angiotensin-induced aldosterone production to $88.6 \pm 2.1\%$ ($n = 35$, Fig. 3a); this slight effect was statistically significant ($P < 0.005$). The corticosterone production rate, estimated in one experiment, was not

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**Fig. 3.** Effect of (a) angiotensin II and (b) ACTH on the production of aldosterone without any inhibitory drug (solid line) or in the presence of indomethacin ($3 \times 10^{-7}$ mol/l; broken line, left-hand panels) or in the presence of eicosatetraynoic acid ($10^{-4}$ mol/l; broken line, right-hand panels). Production rate of aldosterone is expressed as multiples of that by corresponding control incubations without any added substance. Means (± S.E.M.) of 5–8 duplicate incubations are shown.
modified by indomethacin at all (data not shown). As is shown in Fig. 3a, ETY ($10^{-4}$ mol/l) had no effect on aldosterone production rate. In one experiment glomerulosa cells were isolated from indomethacin-pretreated rats, the drug also being added to the medium throughout the preparative and incubation procedures. The response to angiotensin II did not differ from that of control cells (data not shown).

The lowest concentration of ACTH which significantly stimulated the production rate of aldosterone was $3 \times 10^{-10}$ mol/l ($P < 0.02, n = 7$). The effect of ACTH was not modified by indomethacin (Fig. 3b), but the response to high concentrations of ACTH was reduced by ETY ($P < 0.05$ at $3 \times 10^{-8}$ mol ACTH/l and $P < 0.001$ at $3 \times 10^{-7}$ mol ACTH/l) (Fig. 3b). Several other inhibitors of prostaglandin synthesis (niflumic acid, carprofen, meclofenamic acid) and the PGE$_2$ antagonist, 7-oxa-13-prostynoic acid (each at $10^{-4}$ mol/l, $n = 2$ duplicates), did not influence the action of $3 \times 10^{-7}$ mol ACTH/l on aldosterone production.

![Fig. 4](image-url)

Fig. 4. Effect of potassium on aldosterone production without any inhibitory drug (solid line) or in the presence of (a) indomethacin ($3 \times 10^{-4}$ mol/l; broken line) or (b) eicosatetraynoic acid (ETY; $10^{-4}$ mol/l; broken line). Means ($\pm$ S.E.M.) of (a) 4 duplicate incubations and (b) 9 duplicate incubations, respectively, are shown. The dependence of the effect of ETY on the degree of stimulation by potassium is shown in panel (c). The ordinate in panels (a) and (b) and the abscissa in panel (c) indicate the degree of stimulation by potassium. The values represent production rate of aldosterone (Aldo.) expressed as multiples of that by corresponding control incubations ($K^+ = 3.6$ mmol/l). The ordinate in panel (c) indicates aldosterone production by cell suspensions in the presence of ETY divided by that of the matched tubes (i.e. the same concentration of potassium but without ETY).
rate. Neither indomethacin nor ETY influenced the corticosterone production stimulated by ACTH (3 \times 10^{-10} \text{ to } 3 \times 10^{-7} \text{ mol/l}, n = 2 \text{ duplicates}).

Aldosterone production rate, augmented by raised potassium concentration, was not influenced by indomethacin (Fig. 4a). Due to a considerable variation between the responsiveness of control cells to potassium, the conventional presentation of the dose–response curves has masked the effect of ETY (Fig. 4b). It was, however, observed that the effect of ETY depended on the size of the response to potassium. When hormone production was moderately increased, ETY further enhanced the response, while ETY appeared to be inhibitory when steroid production was greatly stimulated by potassium (Fig. 4c).

**DISCUSSION**

Observations in man (Tan & Mulrow, 1977; Norbiato, Bevilacqua, Raggi, Micossi, Moroni & Fasoli, 1978; Speckart, Zia, Zipser, Croxson, Mayeda & Horton, 1978) and in conscious rats (Späth, Józan, Gaál & Mózes, 1977; Späth, Tarján & Tóth, 1979) suggested that prostaglandins were involved in the control of aldosterone synthesis. This assumption was in agreement with a recent report (Shima, Kawashima, Hirai & Asakura, 1980) on the binding of [³H]PGE₁ by the membrane fraction of rat adrenal capsular (glomerulosa) tissue. However, Matsuoka et al. (1980) could not demonstrate the enhancement of aldosterone production by isolated rat glomerulosa cells in the presence of PGE₂ or the prostaglandin precursor, arachidonic acid (3 \times 10^{-8} \text{ to } 3 \times 10^{-4} \text{ mol/l}). Nevertheless, they did not exclude the possibility that the specific prostaglandin receptors had been destroyed during the enzymatic digestion of glomerulosa tissue. In the present work, PGE₂ stimulated aldosterone production by a similar cell preparation and its specific antagonist, 7-oxa-13-prostynoic acid, appeared to inhibit the stimulation in a competitive manner. These findings indicate that no significant deterioration of prostaglandin receptors took place during the cell isolation procedure.

In agreement with recently published observations (Campbell et al. 1980; Miller et al. 1980), arachidonic acid, up to a concentration of 10^{-4} \text{ mol/l}, did not augment the production rate of aldosterone. Above this concentration we observed an enhanced aldosterone production rate but the non-steroidal anti-inflammatory drugs, indomethacin and niflumic acid, failed to overcome this stimulation. Thus, the effect of arachidonic acid may not be regarded as evidence in favour of the aldosterone-stimulating ability of endogenous prostaglandins.

The lack of any significant effect by the non-steroidal anti-inflammatory drugs on basal aldosterone production rate indicates the absence of any essential prostaglandin effect in the non-stimulated state. Differences among published observations (Campbell et al. 1979, 1980; Matsuoka et al. 1980; Miller et al. 1980) in this respect may reflect differences in basal prostaglandin production or in the sensitivity of the isolated cells to non-specific effects of these drugs. The significant augmentation of basal aldosterone production rate by ETY resembles the effect of its counterpart, arachidonic acid, and may be attributed to a non-specific effect of this lipid derivative.

In order to evaluate the mediating or modulating role of prostaglandins in the stimulation of aldosterone production by angiotensin II, potassium ion or ACTH, we have examined the effect of several inhibitors of prostaglandin synthesis on aldosterone production in combination with these substances. Because of the lack of determination of prostaglandins we cannot judge whether there was any significant increase in the production of prostaglandins in response to the agonists applied. Nevertheless, even if the cells were incapable of prostaglandin synthesis, the appropriate increases in aldosterone production upon the addition of its physiological stimulators would imply that production of intracellular prostaglandins is not a prerequisite of a maintained aldosterone production.

There are several reports concerning the effect of prostaglandin inhibitors on angiotensin-
induced hyperaldosteronism but they are by no means unequivocal. Since prostaglandin inhibitors, administered in vivo, may influence the function of the renin–angiotensin system at several points (see Spät et al. 1979), studies with only exogenous angiotensin II and III may be relevant for assessing the role of intra-adrenal prostaglandins. Golub, Speckart, Zia & Horton (1976) reported that in healthy subjects indomethacin did not antagonize the aldosterone-stimulating effect of angiotensin infusion but recalculation of their data might indicate a mathematical error. The aldosterone-stimulating action of angiotensin II was not prevented with indomethacin in hypertensive patients (Frölich, Hollifield, Dormon, Frölich, Seyberth, Michelakis & Oates, 1976). In the rat, indomethacin increased the concentration of angiotensin II and III required to attain half-maximal stimulation of the zona glomerulosa, both in vivo and in vitro. At the same time, meclofenamic acid, another inhibitor of prostaglandin synthesis, failed to modify the effect of angiotensin II (Campbell et al. 1979). In our previous experiments (Spät, 1979) indomethacin reduced both the basal and angiotensin-induced production of aldosterone by rat glomerulosa cells, which were isolated using the method of Haning, Tait & Tait (1970). Simpson, Campanile & Goodfriend (1980) also reported a decreased response to angiotensin II or III by isolated rabbit and bovine glomerulosa cells in the presence of ETY. In the experiments of Miller et al. (1980), both indomethacin and meclofenamic acid abolished the response of rat glomerulosa cells to angiotensin II. However, in the experiments of Spät (1979) and Simpson et al. (1980), as well as of Miller et al. (1980), aldosterone production showed only a twofold increase in response to angiotensin II. In the present experiments the responsiveness of the cells to physiological stimuli was enhanced several times owing to changes in the cell isolation–incubation procedures. In this system indomethacin had but a negligible effect and ETY failed to exert any effect on angiotensin-induced aldosterone production. These findings are in agreement with the recent report of Matsuoka et al. (1980) on the failure of indomethacin to reduce the angiotensin-induced aldosterone production by isolated rat glomerulosa cells. Enhancement of the angiotensin effect by arachidonic acid could indicate the potentiation of angiotensin action by endogenous prostaglandins. Such an enhancement did not, however, take place. On the basis of all these data we doubt that intra-adrenal prostaglandins mediate or modulate the aldosterone-stimulating action of angiotensin II.

In previous experiments in this laboratory (Spät et al. 1979) it was found that indomethacin completely blocked the furosemide-induced, angiotensin-mediated hyperaldosteronism in dexamethasone-pretreated conscious rats while plasma renin activity was still an order of magnitude higher than the control level. It was therefore suggested that indomethacin might interfere with the aldosterone-stimulating action of angiotensin at some adrenal site. However, on the basis of the data presented here, an extra-adrenal site of action of indomethacin seems more likely.

Despite several reports on the stimulatory effect of prostaglandins on steroid production by the adrenal zona fasciculata–zona reticularis (cf. Dupray & Chambaz, 1980a), the role of these substances in mediating or modulating the action of ACTH has not been sufficiently elucidated. Enhanced (Laychock & Rubin, 1975, 1976; Vahouny, Chanderbhan, Hinds, Hodges & Treadwell, 1978), unchanged (Dupray & Chambaz, 1980b; Matsuoka et al. 1980) or even decreased (Ramwell & Shaw, 1970) prostaglandin release by incubated adrenal tissue or cell suspension were observed after adding ACTH. In agreement with the latter observation, increased activity of the catabolic enzymes, prostaglandin-9-oxoreductase and prostaglandin-15-OH dehydrogenase, in response to ACTH was also reported (Levasseur, Friedman & Burke, 1980). Experiments with prostaglandin synthetase inhibitors also resulted in conflicting data. While prostaglandin inhibitors did not reduce the effect of ACTH on glucocorticoid secretion in vivo or production in vitro (Vukosan, Kramer, Pope, Greiner & Colby, 1976; Spät, Sikkös et al. 1977; Beirne & Jubiz, 1978; Hodges, Treadwell & Vahouny, 1978; Matsuoka et al. 1980) dose-dependent as well as time-dependent effects were also observed (Honn & Chavin 1976; Laychock & Rubin, 1976).
The role of prostaglandins in the stimulation of zona glomerulosa by ACTH has been thoroughly investigated. Honn & Chavin (1976) found that indomethacin and 7-oxa-13-prostynoic acid blocked the aldosterone-stimulating effect of ACTH on incubated diced human adrenals in the first 4 min of incubation, but the production rate of hormone did not differ from the control in the subsequent periods. In the rat, indomethacin and aspirin failed to reduce ACTH-enhanced aldosterone production by the incubated glomerulosa tissue (Spät, Siklós et al. 1977). Similar results were obtained from glomerulosa cell suspensions by Matsuoka et al. (1980). The response of adenylate cyclase activity to ACTH and PGE, in the rat glomerulosa tissue was studied by Shima et al. (1980). The maximal stimulation induced by these substances was additive, indicating separate modes of action. Furthermore, a prostaglandin antagonist, polyphloretin phosphate, failed to inhibit ACTH stimulation of adenylate cyclase at a concentration which completely blocked the effect of PGE,1. Miller et al. (1980) reported on a partial inhibition by meclofenamic acid and a complete inhibition by indomethacin of the response to ACTH by rat glomerulosa cells but the response to ACTH without antagonist was only a twofold increase in the production rate of aldosterone.

The present results further substantiate the conclusion that prostaglandins do not play any essential role in the mode of action of ACTH in rat glomerulosa cells. Although ETY, a competitive antagonist of arachidonic acid, reduced the aldosterone-stimulating effect of the high concentrations of ACTH, four different non-steroidal anti-inflammatory drugs, namely indomethacin, niflumic acid, carprofen and meclofenamic acid, as well as the PGE,2-antagonist, 7-oxa-13-prostynoic acid, failed to reduce ACTH-induced aldosterone production rate. The effect of $3 \times 10^{-7}$ mol ACTH/l was also reduced by arachidonic acid ($5 \times 10^{-4}$ mol/l), In this respect the action of arachidonic acid was similar to that of its antagonist, ETY, raising the possibility that depression of the maximal hormone concentration by these compounds is probably not related to intracellular prostaglandin metabolism.

In contrast to our previous observation obtained with incubated glomerulosa tissue (Spät, Siklós et al. 1977) indomethacin failed to reduce ACTH-induced corticosterone production by the glomerulosa cell suspension. Apart from the basic differences in tissue preparation techniques and incubation conditions, we cannot give any explanation for this difference in the corticosterone response.

The most specific stimulators of the adrenal zona glomerulosa are potassium ions. A weak response to potassium ions by rat glomerulosa cells was antagonized by meclofenamic acid and indomethacin in the experiments of Miller et al. (1980). In the present experiments indomethacin did not interfere with the effect of potassium, and the effect of ETY depended on the degree of stimulation. These data indicate that intra-adrenal prostaglandins do not play any essential role in the aldosterone-stimulating action of hyperkalaemia.

The enhancement of aldosterone production in response to arachidonic acid, applied in high concentration, could not be abolished by inhibitors of prostaglandin synthesis. Moreover, arachidonic acid and its antagonist, ETY, had similar effects on basal aldosterone production rate. The degree of stimulation by ACTH or potassium was similarly influenced by the agonist, arachidonic acid, and the antagonist, ETY, suggesting some common features in the mode of action of the two lipid compounds. In this context it should be recalled that arachidonic acid, ETY, 7-oxa-13-prostynoic acid, or non-steroidal anti-inflammatory drugs had comparable effects on the binding of angiotensin by zona glomerulosa membrane preparations (Simpson et al. 1980). Increased turnover of arachidonic acid in adrenal plasma membrane phospholipids is a recently revealed, cyclic AMP-independent rapid response to ACTH (Schrey & Rubin, 1980). Arachidonic acid, unsaturated fatty acids and ETY had a similar effect on the uterine adenylate cyclase system and contractility (Vesin, Khac & Harbon, 1978) suggesting that alterations by fatty acids of membrane architecture affects the adenylate cyclase system and excitation-secretion coupling. On the basis of these observations the possibility that the published effects of
prostaglandin inhibitors on the adrenal cortex were often brought about by aspecific changes in plasma membrane lipid composition cannot be ruled out.

In summary, we have demonstrated that PGE$_2$ specifically stimulated aldosterone production by isolated rat glomerulosa cells, but that prostaglandin inhibitors failed to attenuate the aldosterone-stimulating effect of angiotensin II, ACTH and potassium ions. In view of previously reported data and the present results the importance of intra-adrenal prostaglandins in the physiological control of aldosterone secretion seems to be questionable. Further studies are required to elucidate the biological significance of the prostaglandin sensitivity of adrenal glomerulosa cells.

We are grateful to the suppliers of the materials used in this study.

The effect of non-steroidal anti-inflammatory drugs on prostaglandin production by renal papillary slices was kindly estimated by Dr G. Fejes Tóth in our Department. We thank Dr J. Tamás (Central Chemical Research Institute of the Hungarian Academy of Sciences, Budapest) for the mass spectrometric analysis. The skillful technical assistance of Miss Ibolya Magyar is gratefully appreciated. We thank Dr L. Kiss (Department of Chemical Technology, Technical University, Budapest) for the access to a WANG 2200 computer. The statistical advice of Dr B. Hajtman is gratefully acknowledged.

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Prostaglandins and aldosterone


