ELSEVIER

Molecular and Cellular Endocrinology 101 (1994) 59–66

Molecular and Cellular Endocrinology

Role of specific isoforms of protein kinase C in angiotensin II and lipoxygenase action in rat adrenal glomerulosa cells

R. Natarajan *, L. Lanting, L. Xu, J. Nadler

Department of Diabetes, Endocrinology and Metabolism, City of Hope National Medical Center, 1500 E. Duarte Road, Duarte, CA 91010, USA

(Received 30 September 1993; accepted 6 December 1993)

Abstract

Evidence indicates that the lipoxygenase (LO) pathway of arachidonic acid is a key mediator of angiotensin II (AII)-induced aldosterone synthesis in adrenal glomerulosa cells. Although protein kinase C (PKC) may play a role in AII action, the precise PKC isoforms involved and whether LO products can activate PKC is not clear. We therefore evaluated the effect of AII and LO products such as 12- and 15-hydroxyeicosatetraenoic acids (HETEs) on PKC activation in isolated rat adrenal glomerulosa cells. PKC activity was measured by the phosphorylation of a PKC specific peptide while the PKC isoforms were identified by Western immunoblotting using antibodies that recognize the α, β, γ or ϵ isoforms of PKC. Treatment of the cells for 15 min with AII (10^{-8}M) or the LO products 12- or 15-HETE caused a marked increase in PKC activity in membrane fractions with reciprocal decreases in the cytosolic PKC activity. Rat glomerulosa cells expressed only the α, and ϵ isoforms of PKC. AII increased membrane bound levels of both PKC-α and -ϵ (1.9- and 1.5-fold, respectively), whereas the LO products predominantly activated PKC-ϵ. Reciprocal decreases in immunoreactive cytosolic PKC levels were seen. AII-induced aldosterone synthesis was blocked by H-7 and retinal as well as by a PKC-specific pseudosubstrate inhibitor, PKC(19–36). These results suggest that AII and LO pathway-induced actions in the adrenal glomerulosa may be mediated by specific PKC isoforms.

Key words: Angiotensin II; Protein kinase C; 12-Hydroxyeicosatetraenoic acid; Aldosterone

1. Introduction

Angiotensin II (AII) ia a major regulator of aldosterone synthesis in the adrenal glomerulosa. After binding to its receptor, AII can activate phospholipase C leading to the formation of inositol trisphosphate and diacylglycerol (DAG), and elevation in intracellular calcium due to calcium mobilization (Kojima et al., 1984; Capponi et al., 1984; Fakunding et al., 1979; Catt et al., 1987; Spat, 1988; Braley et al., 1986). Inositol trisphosphate mobilizes intracellular calcium via a specific receptor while DAG functions to activate protein kinase C (PKC) (Kojima et al., 1984; Nishizuka, 1984; Berridge, 1984). It has been suggested that the cellular response to AII is obtained by the temporal integration of the inositol phosphate/calcium-calmodulin branch responsible for the initial transient response and the DAG/PKC branch responsible for the sustained phase (Kojima et al., 1984; Rasmussen and Barret, 1984). Thus in bovine adrenal glomerulosa cells PKC activation by AII has been suggested to be linked to the sustained secretion of aldosterone (Rasmussen and Barret, 1984; Lang and Valloton, 1987). Furthermore, AII-induced activation of the of Na^+-H^+ exchange in adrenal glomerulosa cells is reported to be mediated by PKC (Conlin et al., 1991).

PKC plays pivotal roles in growth and signal transduction and it is now clear that there are at least ten isoforms of PKC which can be classified into two sub-groups, depending on whether they require calcium for activation (PKC-α, -βI, -βII and -γ) or not (PKC-δ, -ϵ, -η(L), -θ, -ζ and -λ) (Nishizuka, 1992; Schaap and Parker, 1990). Considerable interest in the various PKC isoforms arises from the possibility that individual isoforms may have specific and distinct functional roles in the cell. The precise isoforms present in the adrenal glomerulosa have not been clearly defined, although evidence suggests that bovine and rat adrenocortical cells express the α isoform of PKC (Pelosin et

* Corresponding author.

Elsevier Science Ireland Ltd.
SSDI 0303-7207(93)E0303-C

al., 1991). Although it is known that AII can activate PKC in rat adrenal glomerulosa cells (Nakano et al., 1990), the specific isoforms that are activated are not known. Therefore an understanding of the specific isoform(s) that are activated by AII may clarify the precise role of PKC in AII-induced steroidogenic actions in the adrenal.

The DAG arising from AII action can also be metabolised to arachidonic acid by the enzyme DAG-lipase (Bell et al., 1979). Arachidonic acid in the adrenal can be metabolized by the lipoxygenase (LO) pathway to products such as hydroxyeicosatetraenoic acids (HETEs). We have shown that the 12-LO product, 12-HETE, plays a key role in AII-induced aldosterone secretion in rat and human adrenal glomerulosa cells (Nadler et al., 1987; Natarajan et al., 1988). However the mechanism by which 12-HETE promotes the action of AII in the glomerulosa is not fully understood.

In the present study, we have examined whether LO products can increase PKC activity in rat adrenal glomerulosa cells similar to the effect of AII. We have also compared the specific PKC isoforms that are activated by AII as well as the LO products.

2. Materials and methods

2.1. Preparation and incubation of rat adrenal glomerulosa cells

Fresh suspensions of rat adrenal glomerulosa cells were prepared as described earlier (Natarajan and Nadler, 1991). For experiments involving the measurement of aldosterone, the cell suspensions (about 50 000/ml) were incubated with AII (Peninsula Labs, Belmont, CA) for 1 h at 37°C with shaking under an atmosphere of 95% O_2 + 5% CO_2. In some experiments the cells were preincubated for 15 min with protein kinase C inhibitors H-7 or retinal (Sigma Chemical Co., St. Louis, MO). At the end of the incubation a 0.2 ml aliquot was removed for the measurement of aldosterone. Aldosterone was extracted from the cell incubates with methylene chloride and measured by a specific radioimmunoassay using a kit from ICN Biomedicals Inc. (Costa Mesa, CA).

For experiments involving measurement of PKC activity or immunodetection of PKC, cell suspensions (about 1.5×10^6 cells/incubation) were treated for 15 min with agents after a 10 min preincubation at 37°C. The LO products 12- and 15-HETE (Biomol Research Labs., Philadelphia, PA) were added from 1000-fold concentrated solutions in ethanol. The vehicle (0.1% ethanol) was added to the control incubations. Reactions were terminated by cooling on ice and the addition of 10 ml ice-cold phosphate-buffered saline (PBS). Cell pellets were isolated by centrifugation, resuspended in 1.2 ml cold lysis buffer consisting of 20 mM Tris-HCl, pH 7.5. 250 mM sucrose, 0.5 mM EDTA, 0.5 mM EGTA, 1.0 mM dithiothrietol, 10 μM leupeptin, and 10 μM pepstatin and sonicated twice for 5s each at 4°C. The lysate was ultracentrifuged at $100\,000 \times g$ for 1 h and the supernatants saved as the cytosolic fractions. The pellets, after one wash, were resuspended by sonication in 0.16 ml of the lysis buffer containing 0.1% Nonidet P40 and saved as the membrane fraction. Aliquots of the cytosol and membrane fractions were saved for the estimation of protein content. PKC activity and immunodetection of PKC were performed on these subcellular fractions as described below.

2.2. PKC activity

PKC activities in the cytosol and membrane fractions were determined by a PKC assay kit (3161SA) from Gibco BRL (Gaithersburg, MD). The assay is based on the phosphorylation of a synthetic peptide Ac-MBP(4–14) derived from myelin basic protein which was shown by Yasuda et al. (1990) to act as a specific substrate for PKC. PKC specificity is confirmed by using the pseudosubstrate inhibitor peptide PKC(19–36) which acts as potent inhibitor for this substrate (Yasuda et al., 1990; House and Kemp, 1987).

2.3. Immunodetection of PKC

Cytosol and membrane fractions containing equal amounts of protein were subjected to sodium dodecylsulfate (SDS)-polyacrylamide gel electrophoresis and Western blotting as described earlier (Natarajan et al., 1992). The PKC antibodies used were: 1. A polyclonal antibody that recognises the α, β, as well as the γ isoforms of PKC (generous gift from Dr. D. Cooper, University of S. Florida, Tampa); 2. Antibodies that recognize the individual isoforms α, β or γ (generous gifts from Dr. M. Makowske, Memorial Sloan Kettering Center, New York); 3. Antibody to PKC-β (generous gift from Dr B. Roth, Stanford University Medical Center, Stanford) and 4. Antibody to PKC ϵ (generous gift from Dr. B. Strulovic, Institute of Cancer and Developmental Biology, Palo Alto, CA). Immunoblots were scanned with a computerized video densitometer (Applied Imaging Lynx DNA vision, Santa Clara, CA). Absorbance of the major band at 80 kiloDaltons (kDa) was taken as immunoreactive PKC in all cases except for PKC ϵ which appears around 90 kDa (Schaap et al., 1989; Ono et al., 1988).

2.4. Electroporation

Cells used in these experiments were prepared and incubated in a modified Krebs-Ringer bicarbonate

buffer as described (Nadler et al., 1987). Electroporation to facilitate entry of the peptide PKC-specific inhibitor PKC(19–36) (Gibco BRL) was performed with the BRL Cell-Porator™ system. 1 ml aliquots of cell suspensions (about 0.5×10^6 cells) with or without various concentrations of PKC(19–36), were introduced into polypropylene electroporation chambers containing two flat electrodes, 0.4 cm apart, and chilled on ice for 3 min prior to electroporation. The chambers were transferred to the chamber safe and cooled in ice water during electroporation. A single electric pulse (300 V, 800 μF) was delivered. After the discharge, the electroporation chambers were held on ice for 10 min followed by dilution to 3.0 ml. This was divided into three 1 ml aliquots for triplicate incubations. These cell suspensions were preincubated for 10 min at 37°C under an atmosphere of 95% O_2 and 5% CO_2 and the treated with AII for 1 h. Aldosterone was measured in the incubates as described earlier.

3. Data analysis

Results are expressed as mean ± SE from combined experiments as noted in each figure legend. Student's *t*-test and analysis of variance were used to compare control with experimental values and determine significance at a $P < 0.05$ level. For multiple comparisons Dunnet's test was also used. Changes in immunoreactive PKC levels are expressed in arbitrary optical density units obtained from a computerized video densitometer.

4. Results

4.1 The effect of AII and the LO products 12- and 15-HETE on PKC activity

We examined whether AII, 12- and 15-HETE and also the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) can activate PKC in rat adrenal glomerulosa cells by translocating it from the cytosol to the membrane fraction. After incubation of the cells with these agents, PKC enzyme activities were measured in these subcellular fractions. Fig. 1 shows that AII caused a significant increase in the membrane-bound PKC activity at 15 min (control, 89 ± 6 pmol ^{32}P incorporated/min/mg protein vs AII, 108 ± 6, $P < 0.02$, $N = 5$) with a concomitant decrease in cytosolic PKC activity (control, 230 ± 15 pmol ^{32}P vs AII, 121 ± 9, $P < 0.02$, $N = 5$). In addition we also observed that the LO products 12- and 15-HETE at 10^{-7}M could similarly translocate PKC from the cytosol to the membrane fractions suggesting that they also have the capacity to activate PKC in these cells (12-HETE membrane, 114 ± 5 pmol ^{32}P; cytosol, 125 ± 15, both $P < 0.02$ vs corresponding control, $N = 5$; 15-HETE, membrane, 115 ± 8; cytosol, 112 ± 14, both $P < 0.02$ vs control, $N = 4$). The effect of the phorbol ester TPA, a positive control for PKC activation, is also shown in the extreme right panels and it is evident that it depicts similar effects (TPA, membrane, 131 ± 10; cytosol, 70 ± 7, $P < 0.01$ vs control, $N = 4$).

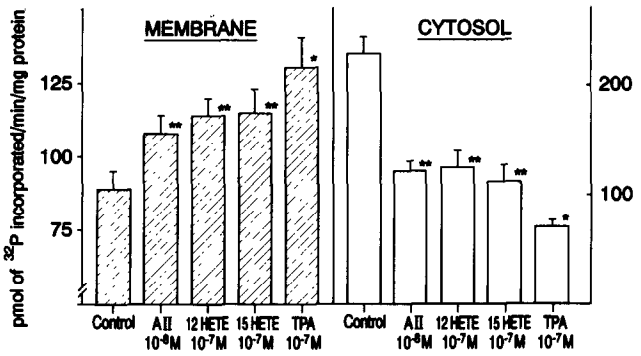

Fig. 1. Subcellular distribution of PKC activity in rat adrenal glomerulosa cells treated with various agents. Cells were treated with the agents for 15 min. Cytosol and membrane fractions were prepared and PKC activity determined as described under Materials and methods. The results are expressed as mean ± SE from 4–5 separate experiments. * $P < 0.01$ vs control; ** $P < 0.02$ vs control (Dunnet's test).

4.2. Activation of PKC isoforms by AII and the LO products

Western immunoblotting using antibodies specific to the α, β, γ or the ϵ isoforms of PKC indicated that rat adrenal glomerulosa cells mainly express the α and ϵ isoforms. We then evaluated the effects of AII, 12- and 15-HETE on immunoreactive α, and ϵ PKC isoform levels in cytosolic and membrane fractions. Fig. 2 shows a representative immunoblot of subcellular fractions obtained from cells treated with agents for 15 min. Blots were probed with an antibody which recognises the α, β, as well as the γ isoforms of PKC. As seen in Fig. 2, in the basal state rat glomerulosa PKC is mainly present in the cytosol and migrated in SDS-polyacrylamide electrophoresis with a molecular mass of nearly 80 kDa. The blot depicts similar results as those obtained for PKC enzyme activity, showing that not only AII, but also both 12- and 15-HETE can cause translocation of immunoreactive PKC from the cytosol to the membrane. The immunoblots were quantitated by densitometry and the arbitrary absorbance units are seen in the bar graph below the blot. This illustrates that the three agents cause nearly a three-fold increase in immunoreactive PKC levels in the membrane fractions and a reciprocal decrease in cytosolic fractions (Membrane: AII, 260% of control; 12-HETE, 290%;

15-HETE, 282%. Cytosol: 37%, 34% and 39% of control, respectively).

We then evaluated whether AII and the LO products could activate the α, and the ϵ isoforms, the two PKC isoforms that we identified in the rat glomerulosa. Fig. 3 shows an immunoblot of subcellular fractions probed with an antibody specific to PKC-α. AII showed a marked increase in immunoreactive PKC-α (80 kDa) levels in the membrane fraction (192% of control) with a concomitant decrease in the cytosol (70% of control). 12-HETE showed no evidence of activating PKC-α (108% and 97%, respectively). 15-HETE treatment did not lead to any increase in membrane-bound PKC-α either, although cytosolic levels did decrease (113% and 65%, respectively).

Fig. 4 shows an immunoblot of subcellular fractions probed with an antibody specific to PKC-ϵ. Immunoreactive PKC-ϵ migrated with a molecular mass of about 90 kDa in SDS-polyacrylamide, as described before (Schaap et al., 1989). The bar graph of the densitometer scan of the blot shows that acute treatment of AII as well as 12- and 15-HETE for 15 min could translocate PKC-ϵ from the cytosol to the membrane fraction,

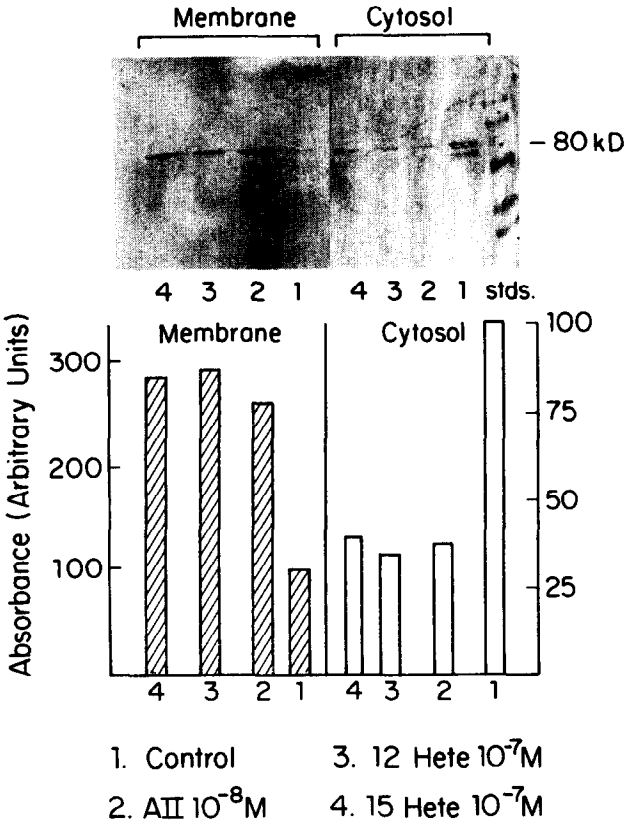

Fig. 2. Immunoblot of subcellular fractions from rat glomerulosa cells probed with a polyclonal antibody which recognises α, β and γ isoforms of PKC. The antibody was used at a dilution of 1:300. The bar graph below represents the densitometric quantitation of the blot in arbitrary optical density units. The results depicted are representative of three separate experiments.

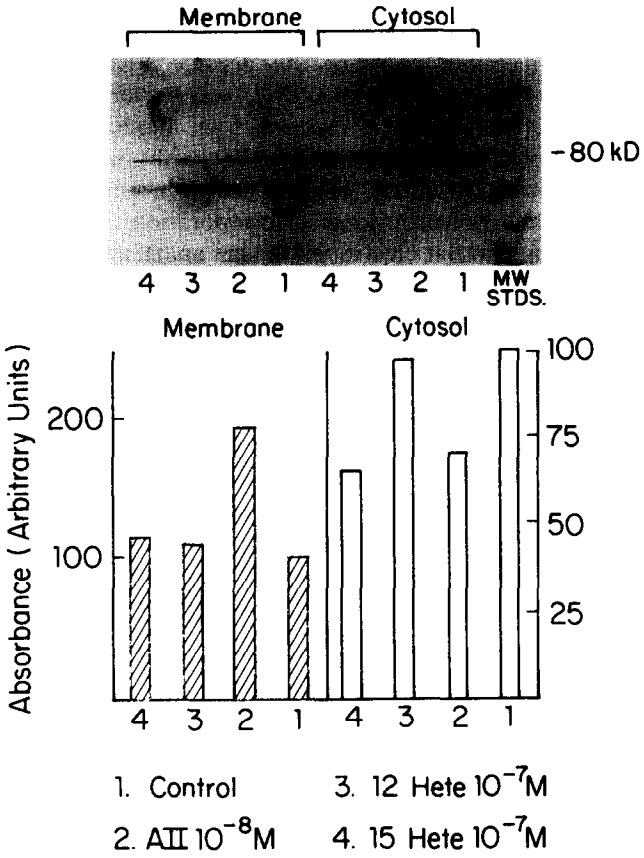

Fig. 3. Immunoblot of subcellular fractions probed with an antibody specific for PKC-α. The antibody was used at a dilution of 1:400. The results depicted are representative of two separate experiments.

thus suggesting that these three agents can activate PKC-ϵ (Membrane: AII, 148% of control; 12-HETE, 126%; 15-HETE, 125%. Cytosol, 66%, 54% and 41%, respectively.)

4.3. Role of PKC inhibitors on AII-induced aldosterone synthesis

In order to determine whether PKC activation may be involved in AII-induced steroidogenesis in rat glomerulosa cells, we initially examined the effect of PKC inhibitors such as H-7 and retinal on AII-induced aldosterone synthesis. As seen in Fig. 5, both of these agents led to a significant inhibition of AII effects suggesting a possible role for PKC in AII-mediated aldosterone synthesis. Both H-7 and retinal at the doses used did not significantly alter basal aldosterone synthesis (results not shown).

We next utilized a highly specific PKC inhibitor peptide, PKC(19–36) to more specifically test the role of PKC in AII action. This 18 amino acid sequence resembling a substrate phosphorylation site is found in the highly conserved amino terminus (regulatory domain) of PKC and functions as a pseudosubstrate for

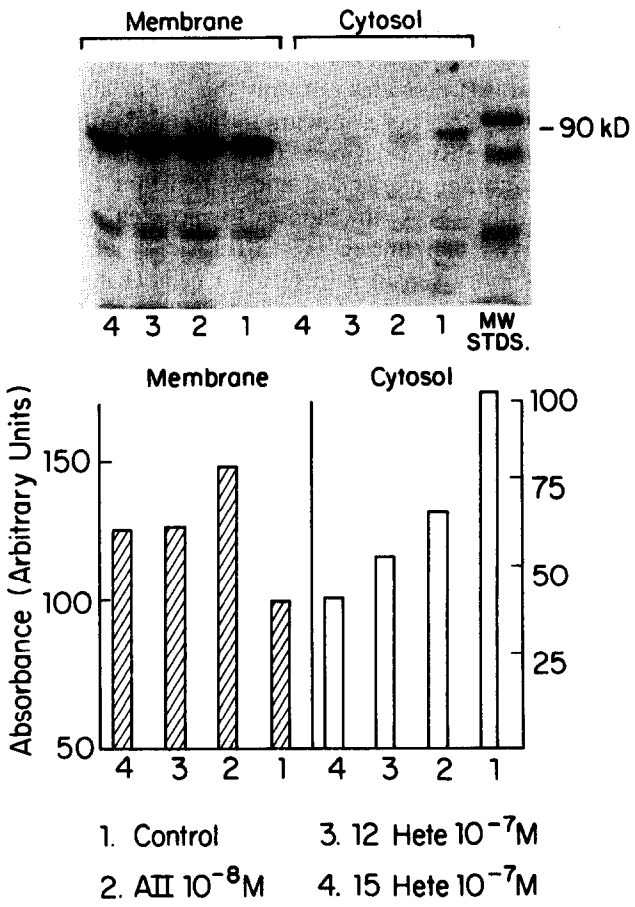

Fig. 4. Immunoblot of subcellular fractions from rat adrenal glomerulosa cells probed with an antibody specific for PKC-ϵ. The antibody was used at a dilution of 1:300. Results shown are representative of two separate experiments.

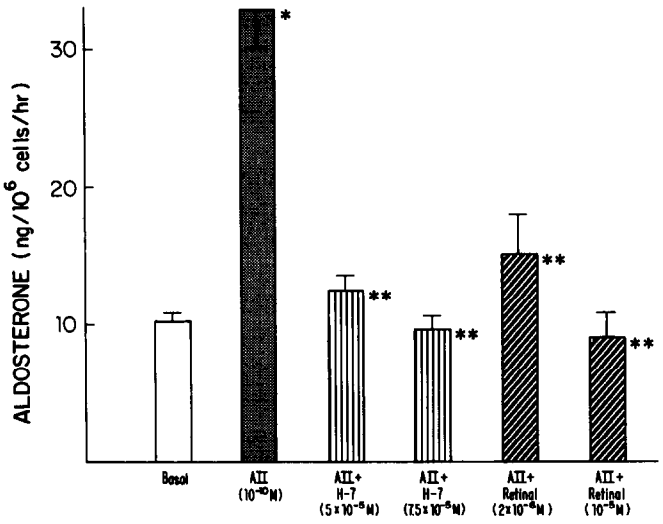

Fig. 5. The effect of PKC inhibitors H-7 and retinal on AII-induced aldosterone synthesis in rat adrenal glomerulosa cells. Results are expressed as mean ± SE from three to four separate experiments performed in triplicate. * $P < 0.001$ vs basal; ** $P < 0.01$ vs AII (Student's t-test).

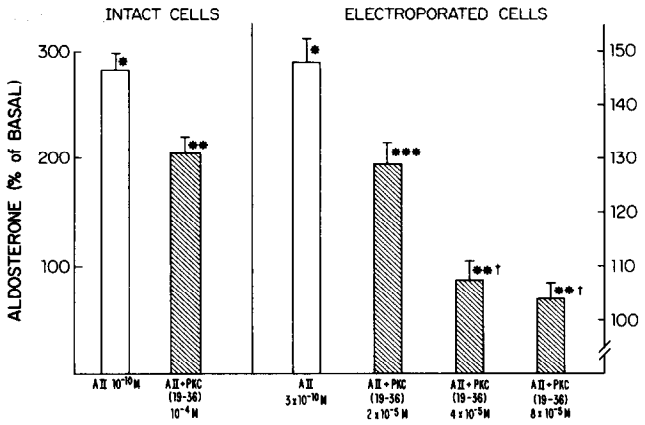

Fig. 6. The effect of PKC(19–36) pseudosubstrate on AII-induced aldosterone synthesis in intact and electroporated rat adrenal glomerulosa cells. The cells were treated for 1 h with AII before of after electroporation as described under Materials and methods. Results are expressed as mean ± SE from three experiments performed in triplicate. * $P < 0.001$ vs basal; ** $P < 0.001$ vs AII; *** $P < 0.01$ vs AII; † $P < 0.01$ vs AII + PKC(19–36)2×10^{-5}M (Student's t-test).

the carboxy terminal catalytic site, thereby acting as an autoregulator of PKC (House and Kemp, 1987). This peptide has been shown to be a highly specific competitive inhibitor of PKC and can therefore be utilized to investigate the role of PKC in hormone action. In Fig. 6, it is seen that even in intact rat glomerulosa cells, PKC(19–36) at 10^{-4}M caused a small but significant inhibition of AII-induced aldosterone synthesis. Due to the poor cellular permeability of this peptide, we examined its effects in electroporated cells. The electroporation process caused some decrease in the potency of AII action, but there was an increased efficacy of PKC(19–36) action in the electroporated cells. Thus Fig. 6 shows that 20 μM PKC(19–36) partially attenuated while 40 and 80 μM concentrations completely blocked AII-induced aldosterone synthesis. PKC(19–36) at concentrations up to 80 μM had no effect on basal aldosterone synthesis (results not shown).

5. Discussion

AII action in the rat adrenal glomerulosa leads to the activation of PKC. Although PKC is suggested to be involved in the steroidogenic effects of AII in bovine adrenal glomerulosa cells (Rasmussen and Barret, 1984; Lang and Valloton, 1987), the role of PKC in AII-induced aldosterone synthesis in rat adrenal glomerulosa cells is not clear (Nakano et al., 1990). Further the specific isoforms of PKC activated by AII are not known. In the present studies we observed that AII caused a significant increase in PKC enzyme activity in the rat adrenal glomerulosa cells. We also used West-

ern immunoblotting with antibodies directed against the α, β, γ and ϵ isoforms of PKC and found that these cells express the α and ϵ isoforms of PKC. Furthermore, AII specifically activated both PKC-α and -ϵ.

AII-induced aldosterone synthesis was attenuated by relatively non-specific PKC inhibitors such as H-7 and retinal as well as by a highly specific pseudosubstrate inhibitor PKC(19–36). It is now known that PKC inhibitors such H-7 and retinal can have non-specific effects in the adrenal and therefore may not yield fully interpretable results (Elliot et al., 1991). Indeed, most of the pharmacological PKC inhibitors were not sufficiently specific for PKC in adrenal cells and many of them, including H-7 and retinal, also attenuated dibutryl cyclic AMP-induced aldosterone synthesis and also had other non-specific effects (Elliot et al., 1991). However, in the present study, we observed that AII effects were also attenuated by a PKC-specific pseudosubstrate inhibitor peptide, PKC(19–36). House and Kemp (1987) showed that PKC contains a pseudosubstrate region (residues 19–31) in its regulatory domain with amino acid sequence resembling a substrate phosphorylation site in its basic residue recognition elements. Synthetic peptide analogs of this sequence such as PKC(19–36) act as potent and specific substrate antagonists. Studies on agonist specificity with this inhibitor peptide in the glomerulosa cells with ACTH and potassium-induced aldosterone synthesis may however also not be clear since PKC has been implicated in the steroidogenic effects of both ACTH and potassium (Cozza et al., 1990; Hajnoczky et al., 1992). In preliminary unpublished experiments, we also observed that in electroporation studies, the PKC(19–36) peptide inhibitor could also attenuate potassium-induced aldosterone synthesis. Thus, although the results from the PKC(19–36) studies may suggest the involvement of PKC in the steroidogenic effects of AII, they cannot give information on specificity and further studies are necessary to resolve the issue of whether PKC mediates AII-induced aldosterone synthesis. However, PKC inhibitors have been shown to attenuate the mitogenic effects of AII in adrenocortical cells (Natarajan et al., 1992). Therefore, it has been suggested that PKC probably plays a role primarily in the chronic effects of AII such as growth responses but does not play a major role in AII-induced aldosterone synthesis in rat adrenal glomerulosa cells since TPA could not mimic the effects of AII (Spat, 1988; Nakano et al., 1990; Vinson et al., 1989) and further, downregulation of PKC by long term TPA treatment did not abolish the aldosterone response to AII in freshly isolated rat glomerulosa cells (Nakano et al., 1990). In contrast, in cultured rat adrenal glomerulosa cells, acute treatment with phorbol dibutyrate directly stimulated aldosterone synthesis (Gallo-Payet et al., 1991). Furthermore, it is now known that TPA downregulates only certain isoforms of PKC without affecting others. In the present study, we observed that AII activates not only the α but also the ϵ isoform of PKC. There appears to be cell-type specific regulation of PKC isozymes. Thus PKC-ϵ is predominantly membrane associated and is resistant to downregulation by TPA in mouse thymocytes and the promonocytic cell line U937 (Strulovic et al., 1991), while it is more sensitive in GH4C1 cells (Kiley et al., 1990). In the present studies, we also observed that rat adrenal glomerulosa PKC-ϵ is mainly present in the membrane fractions. This may explain the persistence of AII effects even during TPA-induced downregulation. Similarly in other cell types the β (Standaert et al., 1993) and the ζ (Ways et al., 1992) isoforms of PKC are not downregulated by TPA pretreatment. Since some of the novel PKC isoforms can act independent of calcium, it is highly likely that these various PKC isoforms can function in parallel to target different sets of intracellular proteins and thereby diversify the cellular response. Further studies are needed to determine whether long term TPA treatment downregulates PKC-δ, -ϵ and/or -ζ in rat glomerulosa cells. But it is evident that chronic TPA treatment studies cannot be conclusively used to evaluate whether PKC activation is required for the steroidogenic response of AII.

We have shown that the LO pathway of arachidonate metabolism plays a key role in AII-induced aldosterone synthesis in rat and human adrenal glomerulosa cells (Nadler et al., 1987; Natarajan et al., 1988). Further DAG was the source of arachidonate for 12-HETE formation (Natarajan et al., 1990). Recently, it was suggested that one of the possible mechanisms of 12-HETE action may be by contribution to the calcium signal generated by AII (Stern et al., 1993). This was based on the observations that LO inhibitors could block the AII-induced calcium signal and that addition of 12-HETE could prevent the inhibition of AII-induced calcium signal by LO inhibitors (Stern et al., 1993).

The results of the current study indicate that these LO products may also mediate steroidogenic or growth responses in the adrenal by the activation of specific PKC isoforms. We observed that the LO products 12- and 15-HETE could directly stimulate PKC enzyme activity in rat glomerulosa cells with the same potency as AII. Further they could specifically activate immunoreactive PKC-ϵ without significantly altering the subcellular distribution of PKC-α. Further studies will be needed to examine whether the HETEs can activate other isoforms such as PKC-δ or -ζ. PKC-ζ is particularly interesting since its recent purification and characterization reveals that it is activated by *cis*-unsaturated fatty acids such as arachidonic acid and linoleic acid (Nakanishi and Exton, 1992). Further, phorbol ester could neither translocate or downregu-

late this isoform (Ways et al., 1992). We also observed that the HETEs could cause translocation of immunoreactive PKC using an antibody which crossreacts with PKC-α, -β, and -γ. Since the HETEs do not activate PKC-α, and rat glomerulosa cells do not appear to express PKC-β and -γ, it is possible that these results were due to the crossreactivity of this antibody with PKC-ζ as has been shown earlier (Cacace et al., 1993). Although 15-HETE could mimic the effects of 12-HETE on PKC, the role of endogenously formed 15-HETE is not yet clear.

Several fatty acids including the HETEs have been shown to activate PKC isolated from the brain (Shearman et al., 1989). We recently demonstrated that 12-LO activation plays a key role in AII-induced mitogenic responses in a bovine adrenal cortical clonal cell line (Natarajan et al., 1992). 12-HETE could increase DNA synthesis in these cells and also directly activate PKC. LO products, including HETEs, have been attributed with mitogenic properties in other cells, such as endothelial cells and various tumor cells. In endothelial cells HETEs act via enhancement of diacylglycerol formation and presumably PKC activity (Shetty et al., 1987), while in tumor cells 12-HETE can induce new receptor formation and metastatic activity via direct activation of PKC (Grossi et al., 1989). The recent observation that PKC-ϵ is an oncogene when overexpressed in rat fibroblasts and leads to malignant transformations (Cacace et al., 1993; Mishak et al., 1993) lends added interest to our current observation that the LO products can activate PKC-ϵ.

The mechanism of how 12-HETE can activate PKC is not very clear. 12-HETE activation of PKC may be a consequence of increasing the intracellular calcium concentration (Stern et al., 1993). However, the activation of PKC-ϵ by the HETEs is not likely to be due to changes in calcium, since PKC-ϵ is a calcium-independent form of PKC. We have preliminary data that 12-HETE can increase DAG mass in rat glomerulosa cells suggesting another potential mechanism for HETE activation of PKC (Wen et al., 1993). Shinomura et al. observed that unsaturated fatty acids and DAG can synergize to activate PKC and suggested that in cells stimulated with agonists which release both DG and fatty acids, PKC once initially activated by the hydrolysis of inositol phospholipids, may sustain its activity even after calcium levels return to basal level since both DAG and unsaturated fatty acids are still available (Shinomura et al., 1991). It has also been shown that the exposure of cells to HETEs causes their rapid incorporation into membrane phospholipids (Legrand et al., 1991; Bandopadhyay et al., 1987) followed by the release of DAG substituted with HETE in position 2, which could activate PKC (Legrand et al., 1991).

These results suggest that specific isoforms of PKC may mediate at least in part the long-term growth and/or short term aldosterone responses to AII and the LO products in rat adrenal glomerulosa cells.

6. Acknowledgements

The authors are deeply grateful to Drs. D. Cooper, M. Makowske, B. Strulovic, and B. Roth for their generous gifts of the PKC antibodies. The authors would also like to thank Dr. Tony Okada for his help with the electroporation experiments.

This work was supported by grants from the National Institutes of Health, R29 HL 48920, RO1 DK39721, and SCOR HL 44404.

7. References

Bandopadhyay, G.K., Imagawa, W., Wallace, D.R. and Nandi, S. (1987) J. Biol. Chem. 263, 7567–7573.

Bell, R.L., Kennerly, D.A., Stanford, N. and Majerus, P.W. (1979) Proc. Natl. Acad. Sci. USA 76, 3238–3241.

Berridge, M.J. (1984) Biochem. J. 220, 345–360.

Braley, L.M., Menachery, A.I., Brown, E.M. and Williams, G.H. (1986) Endocrinology 119, 1010–1019.

Cacace, A.M., Guadagno, S.N., Krauss, R.S., Fabbro, D. and Weinstein, B. (1993) Oncogene 8, 2095–2104.

Capponi, A.M., Lew, P.D., Jornot, L. and Valloton, M.B. (1984) J. Biol. Chem. 259, 8863–8869.

Catt, K.J., Carson, M.C., Hausdorff, W.P., Leach-Harper, C.M., Baukal, A.J., Guillemette G., Balla, T. and Aguilera, A. (1987) J. Steroid Biochem. 27, 915–919.

Conlin, P.R., Williams, G.H. and Canessa, M. (1991) Endocrinology 129, 1861–1868.

Cozza, E.N., Vila, M.D., Acevedo-Duncan, M., Farese, R.V. and Gomez-Sanchez, C.E. (1990) Endocrinology 126, 2169–2176.

Elliot, M.E., Jones, H.M., Tomasko, S. and Goodfriend, T.L. (1991) J. Steroid. Biochem. Mol. Biol. 38, 478–481.

Fakunding, J.L., Chow, R. and Catt, K.J. (1979) Endocrinology 105, 327–333.

Gallo-Payet, N., Chouinard, L., Balestre, M. and Guillon, G. (1991) Endocrinology 129, 623–634.

Grossi, I.M., Fitzgerald, L.A., Umbarger, L.A., Nelson, K.K., Diglio, C.A., Taylor, J.D. and Honn, K.V. (1989) Cancer Res. 49, 1029–1037.

Hajnoczky, G., Varnai, P., Buday, L., Farago, A. and Spat, A. (1992) Endocrinology 130, 2230–2236.

House, C. and Kemp, B.E. (1987) Science 238, 1726–1728.

Kiley, S., Schaap, D., Parker, P., Hsieh, L.L. and Jaken, S. (1990) J. Biol. Chem. 265, 15704–15712.

Kojima, I., Kojima, K., Kreutter, D. and Rasmussen, H. (1984) J. Biol. Chem. 259, 14448–14457.

Lang, U. and Valloton, M.B. (1987) J. Biol. Chem. 262, 8047–8050.

Legrand, A.B., Lawson, J.A., Meyrick, B.O., Blair, I.A. and Oates, J.A. (1991) J. Biol. Chem. 266, 7570–7577.

Mishak, H., Goodnight, G., Kolch, W., Martiny-Baron, G., Schaechtle, C., Kazanietz, M.G., Blumberg, P.M., Pierce, J.H. and Mushinski, J.F. (1993) J. Biol. Chem. 268, 6090–6096.

Nadler, J., Natarajan, R. and Stern, N. (1987) J. Clin. Invest. 80, 1763–1769.

Nakanishi, H. and Exton, J.H. (1992) J. Biol. Chem. 267, 16347–16354.

Nakano, S., Carvallo, P., Rocco, S. and Aguiler, G. (1990) Endocrinology 126, 125–133.
Natarajan, R., Dunn, W.D., Stern, N. and Nadler, J. (1990) Mol. Cell. Endocrinol. 72, 73–80.
Natarajan, R. and Nadler, J. (1991) Endocrinology 128, 2285–2290.
Natarajan, R., Gonzales, N., Hornsby, P.J. and Nadler, J. (1992) Endocrinology 131, 1174–1180.
Natarajan, Stern, N., Hseuh, W., Do, Y. and Nadler, J. (1988) J. Clin. Endocrinol. Metab. 67, 584–591.
Nishizuka, Y. (1992) Science 258, 607–614.
Nishizuka, Y. (1984) Nature 308, 693–698.
Ono, Y., Fuji, T., Ogita, K., Kikkawa, V., Igarashi, K., and Nishizuka, Y. (1988) J. Biol. Chem. 263, 6927–6932.
Pelosin, J., Ricouart, A., Sergheraert, C., Benahmed, M. and Chambaz, E.M. (1991) Mol. Cell. Endocrinol. 75, 149–155.
Rasmussen, H. and Barret, P.Q. (1984) Physiol. Rev. 64, 938–984.
Schaap, D. and Parker, P. (1990) J. Biol. Chem. 265, 7301–7307.
Schaap, D., Parker, P.J., Bristol, A., Kriz, R. and Knopf, J. (1989) FEBS Lett. 243, 351–357.
Shearman, M.S., Naor, Z., Sekiguchi, K., Kishimoto, A. and Nishizuka, Y. (1989) FEBS Lett. 243, 177–180.
Shetty, B.N.Y., Graber, J.E. and Stuart, M.J. (1987) J. Biol. Chem. 262, 17613–17622.
Shinomura, T., Asoaka, Y., Oka, M., Yoshida, K. and Nishizuka, Y. (1991) Proc. Natl. Acad. Sci. USA 88, 5149–5153.
Spat, A. (1988) J. Steroid Biochem. 29, 443–453.
Standaert, M.L., Cooper, D.R., Hernandez, H., Arnold, T.P. and Farese, R.V. (1993) Endocrinology 132, 689–692.
Stern, N., Yanagawa, N., Saito, F., Hori, M., Natarajan, R., Nadler, J. and Tuck, M. (1993) Endocrinology 133, 843–847.
Strulovic, B., Daniel-Issakani, S., Baxter, G., Knopf, J., Sultzman, L., Cherwinski, H., Nestor, J., Webb, D.R. and Ransom, J. (1991) J. Biol. Chem. 266, 168–173.
Vinson, G.P., Laird, S.M., Whitehouse, B.J. and Hinson, J.P. (1989) J. Mol. Endocrinol. 2, 157–165.
Ways, K., Cook, P., Webster, C. and Parker, P. (1992) J. Biol. Chem. 267, 4799–4805.
Wen, Y., Natarajan, R. and Nadler, J. (1993) 75th Annual Meeting of the Endocrine Society, Las Vegas. Abstract No. 1235.
Yasuda, I., Kishimoto, A., Tanaka, S., Tominaga, M., Sakurai, A. and Nishizuka, Y. (1990) Biochem. Biophys. Res. Commun. 166, 1220–1227.