Role of tyrosine kinase and protein kinase C in the steroidogenic actions of angiotensin II, α-melanocyte-stimulating hormone and corticotropin in the rat adrenal cortex

Supriya KAPAS, Andrew PURBRICK and Joy P. HINSON
Department of Biochemistry, Faculty of Medical Sciences, Queen Mary and Westfield College, Mile End Road, London E1 4NS, U.K.

The role of protein kinases in the steroidogenic actions of α-melanocyte-stimulating hormone (α-MSH), angiotensin II (AngII) and corticotropin (ACTH) in the rat adrenal zona glomerulosa was examined. Ro31-8220, a potent selective inhibitor of protein kinase C (PKC), inhibited both AngII- and α-MSH-stimulated aldosterone secretion but had no effect on aldosterone secretion in response to ACTH. The effect of Ro31-8220 on PKC activity was measured in subcellular fractions. Basal PKC activity was higher in cytosol than in membrane or nuclear fractions. Incubation of the zona glomerulosa with either α-MSH or AngII resulted in significant increases in PKC activity in the nuclear and cytosolic fractions and decreases in the membrane fraction. These effects were all inhibited by Ro31-8220. ACTH caused a significant increase in nuclear PKC activity only, and this was inhibited by Ro31-8220 without any significant effect on the steroidogenic response to ACTH, suggesting that PKC translocation in response to ACTH may be involved in another aspect of adrenal cellular function. Tyrosine phosphorylation has not previously been considered to be an important component of the response of adrenocortical cells to peptide hormones. Both AngII and α-MSH were found to activate tyrosine kinase, but ACTH had no effect, observations that have not been previously reported. Tyrphostin 23, a specific antagonist of tyrosine kinases, inhibited aldosterone secretion in response to AngII and α-MSH, but not ACTH. These data confirm the importance of PKC in the adrenocortical response to AngII and α-MSH, and, furthermore, indicate that tyrosine kinase may play a critical role in the steroidogenic actions of AngII and α-MSH in the rat adrenal zona glomerulosa.

INTRODUCTION

Angiotensin II (AngII) and corticotropin (ACTH), the two major stimuli of aldosterone secretion, act through two distinct signal-transduction pathways. ACTH stimulates adenylate cyclase activity thereby increasing cyclic AMP which in turn activates cyclic AMP-dependent protein kinase [1,2]. In the rat adrenal zona glomerulosa, α-melanocyte-stimulating hormone (α-MSH) shares a common mechanism of action with AngII; both peptides activate phospholipase C resulting in the formation of inositol 1,4,5-trisphosphate which raises intracellular Ca^{2+} and diacylglycerol concentrations, which activates protein kinase C (PKC) [3–5].

PKC, originally isolated from rat brain cytosol, has been found to mediate a wide range of cellular responses [6]. Studies have shown that cell stimulation by agents known to activate phospholipase C is followed by translocation of PKC from the cytosol to the membrane [6–8], and it is at the plasma membrane that PKC is activated by diacylglycerol [9].

The mechanism of signal transduction at the plasma membrane level appears to be well understood whereas events associated with the cell nucleus are less clear. It was initially documented that PKC is either absent from or poorly represented in the nucleus [6]. However, further studies suggest that PKC phosphorylates a number of nuclear proteins and may be involved in the regulation of transcription and expression of proto-oncogenes such as c-*fos*, c-*jun* and c-*ref-1* [10–15]. Previous studies from this laboratory showed that effects such as AngII and α-MSH cause translocation of PKC from the cytosol to the membrane [16]; however, their effects on nuclear PKC have not been investigated. In the present study experiments were carried out to elucidate the role of nuclear PKC in rat adrenal steroidogenesis.

Tyrosine kinase activity and tyrosine phosphorylation of cellular proteins are usually considered to be fundamental elements of the mitogenic response to various growth factors, particularly epidermal growth factor [17–19]. Kinase activity usually results in the phosphorylation of cellular proteins which may then be activated [20–22]. There has been some speculation that tyrosine kinase activation is secondary to activation of PKC in some tissues. PKC has been postulated, on the basis of observations that phorbol ester alone can activate tyrosine kinase and enhance tyrosine phosphorylation [23–25], to play an important role. There have been no reports to date on the possible role of tyrosine kinase in response to AngII, α-MSH or ACTH on the rat adrenal zona glomerulosa *in vitro*.

These experiments were designed to examine the roles of PKC and tyrosine kinase in AngII-, α-MSH- and ACTH-stimulated aldosterone secretion using the antagonists Ro31-8220, a potent selective PKC inhibitor [26,27] and tyrphostin 23, a specific inhibitor of tyrosine kinases [28].

EXPERIMENTAL

Materials

α-MSH, ACTH(1–24) (Synacthen) and AngII amide (Hypertensin) were obtained from Ciba-Geigy, Horsham, Sussex, U.K.; collagenase (Worthington type I) was from Lorne Diagnostics, Reading, Berks., U.K.; the PKC inhibitor Ro31-8220 was obtained from Roche Laboratories, Welwyn Garden City, Herts.,

Abbreviations used: AngII, angiotensin II; ACTH, corticotropin; α-MSH, α-melanocyte-stimulating hormone; PKC, protein kinase C; KRBG, Krebs–Ringer bicarbonate buffer with glucose; PMA, phorbol 12-myristate 13-acetate.

U.K.; Raytide (peptide substrate for tyrosine kinase) was from Cambridge Bioscience, Cambridge, U.K.; tyrphostin 23, genistein and calphostin C were obtained from LC Laboratories, Calbiochem-Novabiochem Ltd., Nottingham, U.K. All radiolabels were obtained from Amersham International plc, Amersham, Bucks., U.K. All other chemicals were of analytical grade and obtained from Sigma Chemical Co., Poole, Dorset, U.K. or BDH, Dagenham, Essex, U.K.

Preparation of whole rat adrenal glomerulosa tissue

Male and female Wistar rats (250–400 g body weight), supplied by A. Tuck and Sons, Battlesbridge, Essex, U.K. and maintained at Queen Mary and Westfield College, were used. The rats were stunned then killed by cervical dislocation. Adrenals were rapidly removed and cleaned of adhering fat. Capsule fractions (containing mainly glomerulosa cells) were separated from inner adrenocortical tissue by pressure between glass plates. Adrenal capsules were incubated in 5 ml of Krebs–Ringer bicarbonate containing glucose (200 mg/100 ml) (KRBG) for 60 min at 37 °C under an atmosphere of 95% O_2 and 5% CO_2. After 60 min preincubation, the medium was discarded and fresh KRBG was added. Capsules were then incubated in 1 ml of KRBG for 2 h under an atmosphere of 95% O_2 and 5% CO_2 in the absence or presence of stimulants and inhibitors.

Determination of the effect of protein kinase inhibitors on peptide-stimulated steroidogenesis

All peptides were dissolved in KRBG to the required concentration. Ranges of concentrations of ACTH from 10^{-13} to 10^{-7} M, α-MSH from 10^{-11} to 10^{-6} M and AngII from 10^{-11} to 10^{-6} M were used in the presence or absence of Ro31-8220 (150 nM), calphostin C (50 nM), tyrphostin 23 (50 nM) or genistein (500 nM). After a 60 min incubation period, capsules were discarded and media decanted into Eppendorf tubes. Tubes were stored at −20 °C until the media were ready to be assayed. Aldosterone was measured in a portion of unextracted incubation medium by direct radioimmunoassay [16].

Preparation of adrenal subcellular fractions

Adrenal capsules were obtained and treated as described above. After incubation, tissue was homogenized using a Polytron. This required three to four passes of 15 s at setting 5 with 1 min intervals, keeping the tissue on ice, in 15 vol. of buffer A [250 mM sucrose, 20 mM Tris/HCl (pH 7.5), 2 mM EDTA, 0.5 mM EGTA, 0.1% (v/v) 2-mercaptoethanol, 0.02 mM leupeptin and 1 mM phenylmethanesulphonyl fluoride]. Homogenates were then subjected to differential centrifugation. Crude nuclei were sedimented at 800 *g* and washed and re-centrifuged twice with buffer A containing 30% glycerol. The 800 *g* supernatant was centrifuged at 100000 *g* for 20 min. The supernatant was considered as the cytosolic fraction and the pellet as membrane. Nuclear preparations were stained and studied under light microscopy and were found to be relatively uncontaminated with other cellular components (results not shown).

Determination of PKC activity

PKC activity was assayed by estimating the incorporation of ^{32}P from labelled ATP into type-III histone substrate. Briefly, whole adrenal capsules were obtained as described above and preincubated in 5 ml of KRBG for 60 min. The preincubation media were then discarded and the capsules incubated in fresh KRBG with increasing concentrations of AngII, α-MSH or ACTH in the presence or absence of kinase inhibitors for 60 min. PKC activity was measured as described by Persaud et al. [29] and modified by Vinson et al. [30]. The standard assay buffer contained 20 mM Hepes (pH 7.5) with 24 mM $CaCl_2$, 200 mM magnesium acetate, 5 mg/l histone, 216 μg of phosphatidylserine/assay tube, 15 μg of diolein/assay tube and [γ-^{32}P]ATP (3000 Ci/mmol; 1 μCi/assay tube). An assay volume of 20 μl was combined with 20 μl of test sample containing 5–20 μg of protein and the assay tubes were incubated for 20 min at 30 °C. BSA (50 μl; 10 mg/ml) was added to each tube and protein precipitated by adding 10% trichloroacetic acid. Precipitate was collected, washed on Millipore filters and the isotope content was measured. Protein content of enzyme preparations was determined by the method of Lowry et al. [31].

Peptide assay of tyrosine kinase activity

The basis of the assay is the separation of the ^{32}P-labelled peptide substrate from unchanged [^{32}P]ATP by adsorption of the former to phosphocellulose paper at low pH [32]. Whole adrenal capsules were obtained as described above and preincubated in 5 ml of KRBG for 60 min. The preincubation media were then discarded and the capsules incubated in fresh KRBG with increasing concentrations of AngII, α-MSH or ACTH in the presence or absence of Ro31-8220 or tyrphostin 23 for 60 min. After incubation, the media were discarded and the capsules homogenized in kinase assay buffer [50 mM Hepes (pH 7.5), 100 μM EDTA, 0.01% Triton X-100, 100 μM sodium orthovanadate] and resuspended to give a concentration of 1.5 mg of protein/ml.

In order to measure maximal kinase activity the effects of increasing time and enzyme concentration on ^{32}P incorporation were assessed. Incorporation of ^{32}P into peptide substrate was linear with time and enzyme concentration up to 50 min and 40 pmol/min per mg of protein respectively. Assay blanks were also run and the ratios of sample values versus blank values were generally 40:1. Phosphorylation assays were carried out in a small volume of kinase dilution buffer (kinase buffer containing 0.75 mg of Raytide/ml, 0.3 mg of BSA/ml, 3 mM dithiothreitol). For each reaction, 10 μl of kinase dilution buffer was combined with 10 μl of homogenate on ice. The reaction was started by the addition of 10 μl of ATP mixture [assay buffer plus 30 mM $MgCl_2$ and 200 μCi/ml [γ-^{32}P]ATP (3000 Ci/mmol)] and brief vortexing. The reaction mixture was incubated for 30 min at 30 °C. The reaction was stopped by the addition of 120 μl of 10% (w/v) phosphoric acid and vortexing. Then 120 μl of each mixture was pipetted on to 2.5 cm × 2.5 cm squares of Whatman P81 phosphocellulose paper. The P81 papers were extensively washed with 0.5% (w/v) phosphoric acid (4 × 1 litre for 50 papers) followed by a final wash in acetone. They were then left to dry in air, transferred to scintillation vials and their isotope contents measured. The protein content of the homogenates was estimated by the method of Lowry et al. [31].

Statistical analysis

Arithmetic means and S.E.M. values were calculated, usually for $n = 4$, i.e. the results from four separate experiments were combined. One-way analysis of variance was used to test whether AngII, α-MSH or ACTH had a significant effect on basal (control) levels of aldosterone, PKC or tyrosine kinase activity. Student's *t* tests were used to test whether the protein kinase inhibitors had any effect on agonist-stimulated events compared with agonist alone.

RESULTS

Effects of inhibitors on steroid secretion

AngII, α-MSH and ACTH caused dose-dependent increases in aldosterone production from the rat adrenal glomerulosa (Figures 1a–1c). Threshold stimulation of aldosterone secretion was 10^{-10} M for AngII and α-MSH and 10^{-2} M for ACTH.

Dose-dependent inhibitory studies were carried out to determine the optimal concentration of the inhibitors to use in this study (results not shown). It was ascertained that Ro31-8220 should be used at 150 nM, calphostin C at 50 nM, tyrphostin 23 at 50 nM and genistein at 500 nM. Figure 1 shows that the actions of AngII and α-MSH on aldosterone production were significantly attenuated by the PKC inhibitors Ro31-8220 and calphostin C ($P < 0.001$) causing up to 75% inhibition. These inhibitors had no effect on ACTH-stimulated aldosterone secretion. Phorbol 12-myristate 13-acetate (PMA; 10^{-9} to 10^{-5} M) also stimulated aldosterone production which was significantly inhibited by Ro31-8220 and calphostin C (results not shown).

The protein tyrosine kinase inhibitors tyrphostin 23 and genistein, at a concentration of 50 nM and 500 nM respectively, had no effect on aldosterone secretion in response to ACTH (Figure 1c). However, tyrphostin 23 did cause significant attenuation of AngII- and α-MSH-induced steroid output from rat zona glomerulosa *in vitro* (Figures 1a and 1b) by lowering the maximum aldosterone response to both these stimulants.

Table 1 Subcellular distribution of Ca^{2+}/phospholipid-sensitive PKC in the rat adrenal glomerulosa

The effects of various protein kinase inhibitors on AngII-, α-MSH- and ACTH-stimulated PKC activity are reported. Ro, 150 nM Ro31-8220; Tyr, 50 nM tyrphostin 23; Cal, 50 nM calphostin C; Gen, 500 nM genistein. Values (expressed as means ± S.E.M.; $n = 3$) show ^{32}P incorporation into type-III histone substrate from [^{32}P]ATP. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control (analysis of variance); †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared with peptide hormone alone (Student's t test).

	PKC activity (% of total)		
Pretreatment	Cytosol	Membrane	Nucleus
None (control)	80±8	12±12	8±8
AngII (10^{-8} M)	38±25***	25±4***	37±3***
AngII + Ro	79±6†††	10±3†	11±2†††
AngII + Tyr	55±4†††	25±3	20±4†
AngII + Cal	72±5†††	15±2B	13±1†††
AngII + Gen	60±5†††	20±2	20±3††
α-MSH (10^{-8} M)	12±2***	65±6***	23±3***
α-MSH + Ro	62±6†††	23±3†††	15±2†††
α-MSH + Tyr	40±5†††	55±2†	5±1†††
α-MSH + Cal	70±6†††	20±2†††	10±2††
α-MSH + Gen	40±5†††	56±2†	10±2††
ACTH (10^{-9} M)	58±6*	25±5	17±2**
ACTH + Ro	64±5	26±3	10±2†
ACTH + Tyr	61±5	26±3	13±2
ACTH + Cal	60±5	25±2	15±2
ACTH + Gen	64±5	21±3	15±2

Effects of inhibitors on PKC activity

In control (unstimulated) tissue, PKC activity was mainly found in the cytosolic fraction, about 80%, with 12% and 8% in the membrane and nuclear fractions respectively (Table 1).

The effects of AngII, α-MSH and ACTH stimulation on the subcellular distribution of PKC activity in whole glomerulosa tissue are shown in Table 1. Both membrane- and nuclear-associated PKC activities were significantly increased after incubation with 10^{-8} M AngII and cytosolic activity was decreased compared with control. A similar translocation of PKC activity was seen with 10^{-8} M α-MSH. ACTH had no effect on membrane-associated PKC. However, it did cause a decrease in cytosolic PKC and a significant increase in nuclear PKC activity ($P < 0.05$).

To study the effects of inhibitors on PKC activation, tissue was exposed to 150 nM Ro31-8220, 50 nM calphostin C, 50 nM tyrphostin 23 or 500 nM genistein before incubation with the various stimulants. The PKC inhibitors, Ro31-8220 and calphostin C, caused significant decreases in membrane and nuclear PKC activity in response to either AngII or α-MSH and a corresponding increase in cytosolic enzyme activity (Table 1).

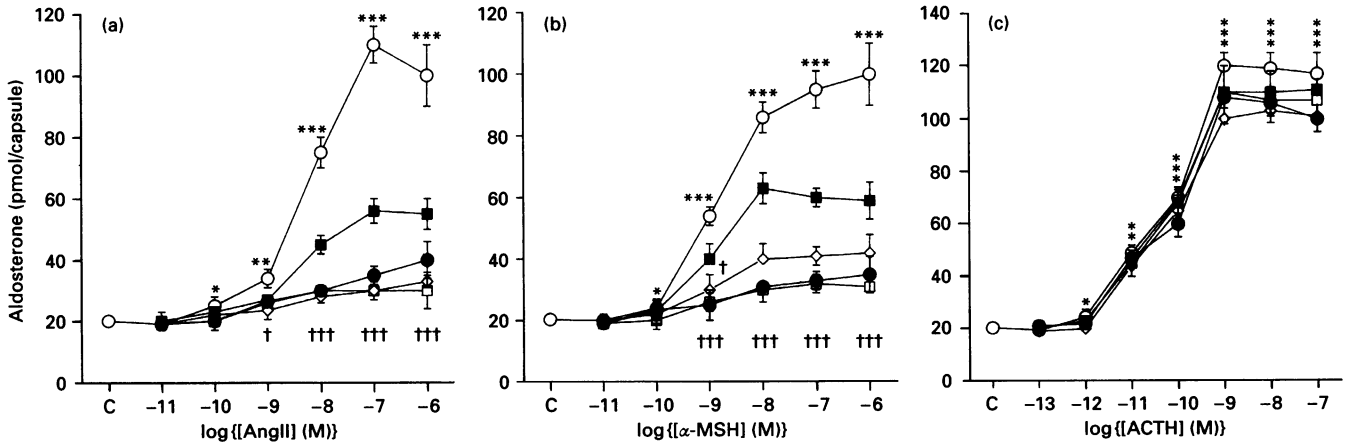

Figure 1 Effect of kinase inhibitors on peptide-hormone-stimulated aldosterone secretion

The effects of increasing concentrations of (**a**) AngII, (**b**) α-MSH and (**c**) ACTH on aldosterone secretion from whole rat capsules in the absence (○) and presence of 50 mM tyrphostin 23 (●), 500 nM genistein (□), 150 nM Ro31-8220 (■) or 50 nM calphostin C (◇) are shown. C, Control. Values are means ± S.E.M. ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with basal levels of aldosterone (analysis of variance); †$P < 0.05$, †††$P < 0.001$ compared with agonist alone (Student's t test).

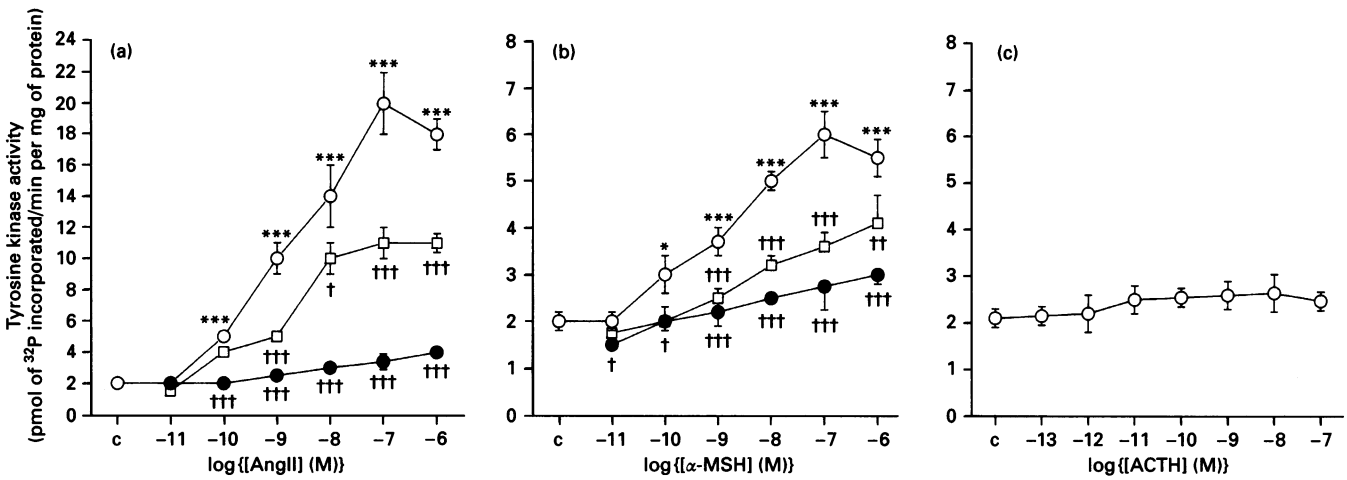

Figure 2 Effect of Ro31-8220 and tyrphostin on peptide hormone-stimulated tyrosine kinase activity

The effects of increasing concentrations of (**a**) AngII, (**b**) α-MSH and (**c**) ACTH on protein tyrosine kinase activity in the absence (○) and presence of 50 nM tyrphostin 23 (●) or 150 nM Ro31-8220 (□) are shown. C, Control. Values (means ± S.E.M.; $n = 4$) show ^{32}P incorporation into tyrosine kinase substrate. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with control (analysis of variance); $\dagger P < 0.05$, $\dagger\dagger P < 0.01$, $\dagger\dagger\dagger P < 0.001$ compared with agonist alone (Student's t test).

In other words, Ro31-8220 and calphostin C prevented translocation of PKC activity in response to AngII or α-MSH. The increase in nuclear PKC activity in response to ACTH was also inhibited by Ro31-8220.

Table 1 also illustrates the effect of the tyrosine kinase inhibitors, tyrphostin 23 and genistein, on the subcellular distribution of PKC in rat glomerulosa tissue stimulated with AngII, α-MSH or ACTH. Both inhibitors caused a significant reduction (30–50%) in AngII- and α-MSH-stimulated nuclear PKC activity associated with a similar increase in cytosolic PKC activity. The tyrosine kinase inhibitors had no effect on ACTH-stimulated nuclear PKC.

Effects of inhibitors on tyrosine kinase activity

The ability of these stimulants to activate tyrosine kinase was studied using Raytide as a substrate for the kinase assays. All kinase assays were carried out with a substrate concentration of 0.25 mg/ml which was found to give optimal activity (results not shown). Figure 2 shows ^{32}P incorporation by tyrosine kinase. AngII and α-MSH dose-dependently stimulated tyrosine kinase activity. Threshold stimulation was at 10^{-10} M for AngII and α-MSH (Figure 2a and 2b). The actions of AngII and α-MSH on tyrosine kinase activity were significantly ($P < 0.001$) inhibited by the protein tyrosine kinase inhibitor tyrphostin 23. ACTH did not stimulate tyrosine kinase activity at any concentration used (Figure 2c).

The PKC inhibitor Ro31-8220 also caused significant attenuation of AngII- and α-MSH-induced tyrosine kinase activity from rat glomerulosa *in vitro* (Figures 2a and 2b). Genistein and calphostin C also inhibited AngII- and α-MSH-induced tyrosine kinase activity (results not shown) although these antagonists were less effective than tyrphostin 23.

DISCUSSION

The present study provides new insights into the role of protein kinases in aldosterone secretion stimulated from the rat zona glomerulosa. When PKC was inhibited by either Ro31-8220 or calphostin C, AngII- and α-MSH-induced aldosterone production was considerably decreased (Figures 1a and 1b). These inhibitors had no effect on ACTH-stimulated aldosterone secretion. These data confirm our previous findings, indicating the importance of the PKC-signalling pathway in aldosterone output in response to AngII or α-MSH, but not ACTH. The results are different from those obtained by other workers [33,34] who reported that PKC did not play an important role in AngII-stimulated steroid production. The reasons for this discrepancy are not entirely clear; however, differences in experimental procedure may account for part of the difference. The use of whole zona glomerulosa tissue appears to be an important factor in observing the PKC-dependent aspect of aldosterone secretion [30]. In the present study, AngII induced a more than 5-fold increase in aldosterone production, whereas other workers did not obtain this [33,34]. The latter based their conclusions on observations of down-regulation of PKC by pretreating cells with phorbol ester, but recent reports [35,36] have shown that pretreatment of adrenocortical cells with phorbol esters may have other non-specific effects.

When PKC activity itself was studied, it was interesting to discover enzyme activity in the nucleus. Little progress has been made towards characterization of protein kinases that act at the nucleus, although a number of nuclear serine and theronine kinases are documented [37–40]. There is conflicting evidence about nuclear PKC. Nishizuka [6] negated the possibility of PKC being located in the nucleus on the basis of immunocytochemical analysis. However, there is evidence to suggest a nuclear role for PKC, for instance phosphorylation of nuclear proteins [10,39,41]. In view of mounting evidence for a functional role of PKC at the nucleus, two aspects are worth considering. First, is PKC translocated from the cytosol to the nucleus, and secondly, is there endogenous PKC present in the nucleus? The present study supports both possibilities. Table 1 shows that, under basal conditions in the zona glomerulosa, 80, 12 and 8% of PKC activity was located in the cytosol, membrane and nuclear fractions respectively, in agreement with other observations [42]. On stimulation with AngII, α-MSH or PMA (results not shown), the intracellular distribution of PKC changed dramatically,

causing significant increases in nuclear and membrane PKC associated with a decrease in cytosolic PKC.

Treatment with ACTH did not alter membrane content of PKC, but nuclear PKC activity was enhanced. However, it is evident that translocation of PKC to the nucleus is not important for the steroidogenic effects of ACTH as the PKC inhibitors, Ro31-8220 and calphostin C, had no effect on ACTH-stimulated aldosterone secretion. PKC has been reported to be involved in the regulation of transcription factors [12,14,40,43,44]. It is possible to speculate on the mechanism by which receptor-triggered signal transduction induces transcription of regulatory factors. Recent reports have suggested that ACTH is capable of stimulating protein kinases, PKC in particular, which may be involved in transmitting the hormonal signal to the nucleus to regulate genes such as c-*fos* and c-*jun* in adrenal glands [45–47].

In the study presented here, a novel biochemical signalling pathway in the rat adrenal zona glomerulosa has been identified. AngII and α-MSH stimulated tyrosine kinase activity in a dose-dependent manner, whereas ACTH did not (Figure 2). Specific tyrosine kinase inhibitors, tyrphostin and genistein, caused almost complete abolition of tyrosine kinase activity in the zona glomerulosa in response to these peptide hormones, and, furthermore, inhibited steroidogenesis. It has been reported that AngII can stimulate tyrosine kinase in other cell types such as rat aortic smooth-muscle cells and glomerular mesangial cells [25,48,49]. In these cells, AngII has a significant mitogenic effect which appears to be mediated by tyrosine phosphorylation. These workers also suggest that PKC activation is an important intermediate step necessary for tyrosine kinase activation. Further evidence for this observation comes from studies carried out using phorbol esters. Experiments with both PKC inhibitors and depletion demonstrated that PKC was necessary but not sufficient for tyrosine kinase activity [44,49,50]. Sequential activation of intracellular protein kinases has been associated with signal transduction in a number of tissues [49–53]. Although Ro31-8220 is a specific inhibitor of PKC activity, it also had a significant inhibitory effect on AngII- and α-MSH-induced tyrosine kinase activity (Figure 2). This suggests that PKC may trigger activation of tyrosine kinase. Despite the apparent blocking of the PKC-dependent pathway by Ro31-8220, agonist-stimulated tyrosine kinase activity was not totally inhibited, suggesting that there may be a PKC-independent pathway of tyrosine kinase activation.

The role of individual PKC isoforms in cells cannot be identified using PKC antagonists that block the activity of more than one isoenzyme. Ro31-8220 is known to be specific for PKC rather than other protein kinases [26]. In addition Ro31-8220 preferentially inhibits PKCα over PKCβ, γ and ε [27,54]. The determination of PKC isoforms in the rat adrenal gland is unclear and requires thorough investigation. However, data presented here support the view that PKC is probably an important part of the signalling pathway activating tyrosine kinases in response to phospholipase C-linked agonists in the rat adrenal zona glomerulosa.

The precise mechanisms responsible for functional coupling of the activated AngII receptor and tyrosine kinase activity are at present unknown. The rat adrenal zona glomerulosa tissue used in this study expresses the AT_1-type receptor [55–59]. The receptor has recently been cloned [60,61] and it appears not to have any intrinsic tyrosine kinase activity. Therefore the binding of AngII to the AT_1 receptor may rapidly induce additional coupling to intracellular tyrosine kinases. A recent study by Hausdorff et al. [62] has shown that *src*-tyrosine kinase can phosphorylate the α-subunit of many trimeric G-proteins *in vitro*.

In summary, we have characterized an additional signalling mechanism stimulated by AngII and α-MSH in the rat adrenal zona glomerulosa. Further studies aimed at identification of activated intracellular protein tyrosine kinases and defining the precise role of PKC in agonist-induced aldosterone secretion are being carried out. The results may provide a more complete understanding of the integrated cellular function of phospholipase C-coupled hormones in the rat adrenal cortex.

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