Production of platelet-activating factor is a component of the angiotensin II–protein kinase C activation pathway in bovine adrenocortical cells

Jean Marc PELOSIN, Michelle KERAMIDAS and Edmond M. CHAMBAZ*

DBMS/LBIO/BRCE, INSERM U244, CEN.G, BP85X, 38041 Grenoble, Cedex, France

Lyso-platelet-activating factor (lyso-PAF): acetyl-CoA acetyltransferase (EC 2.3.1.67) enzyme activity was characterized for the first time in bovine adrenocortical tissue. It was found to be associated with the microsomal membrane fraction, in which it exhibited a specific activity of 0.4 nmol/min per mg of protein and catalytic properties similar to those described in other cell types. The adrenocortical acetyltransferase activity was increased by 2–3-fold on incubation of the preparation with purified protein kinase C (PKC) under phosphorylating conditions. This activation was optimal after 5 min of incubation and paralleled an increase in PKC-catalysed ^{32}P incorporation into microsomal proteins. Both acetyltransferase activation and protein phosphorylation were dependent on the presence of Ca^{2+} and phospholipids, and were blocked in the presence of the potent PKC inhibitor H-7. In the intact adrenocortical cell, angiotensin II and a potent phorbol ester (phorbol 12-myristate 13-acetate) were able to rapidly induce an increase in the biosynthesis of PAF, which was mostly released into the extracellular medium. These data suggest that bovine adrenocortical lyso-PAF acetyltransferase may be regulated by a PKC-dependent activation pathway, whereas no evidence for an additional adrenocorticotropin/cyclic AMP-dependent stimulation process was obtained in this cell type. Bovine adrenocortical cell membrane preparations were shown to possess high-affinity PAF-binding sites ($K_d \sim 0.5 \text{ nM}$). Altogether, these observations suggest that PAF production and release may play a role in the autocrine or paracrine control of adrenocortical cell activation.

INTRODUCTION

Platelet-activating factor (PAF) was first identified as a phospholipid mediator involved in cellular inflammatory responses [1]. More recently, however, a number of different cell types such as fibroblasts [2] and endothelial cells [3] have been shown to synthesize PAF in response to various stimuli, and this ether phospholipid has been suggested to be a cellular messenger of wide significance (for a review, see [4]). The biosynthetic pathway of PAF involves two steps: (i) deacylation of acyl-alkylglycerophosphocholine by a phospholipase A_2 , resulting in the release of lyso-PAF; and (ii) transfer of an acetyl moiety from acetyl-CoA to lyso-PAF, catalysed by 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine:acetyl-CoA acetyltransferase (EC 2.3.1.67).

A lyso-PAF acetyltransferase activity has been described in various tissues but, to our knowledge, the enzyme has been obtained only in a partially purified form [5,6]. It is believed to represent a control point in the biosynthesis of PAF. Regulation of the acetyltransferase activity by a phosphorylation/dephosphorylation process has been documented; a positive effect of a cyclic AMP-dependent phosphorylation [5,6], as well as the suggestion of activation through phosphorylation by protein kinase C (PKC) [7,8], have been reported.

To our knowledge, PAF has not yet been examined as a possible endogenous component of the signalling systems involved in the control of steroidogenic adrenocortical cell functions, although it has been reported in preliminary form that exogenous PAF may activate adrenocortical cell steroidogenesis [9]. Bovine adrenocortical cells acutely increase their cortisol

production following stimulation by two major physiological peptides, i.e. adrenocorticotropin (ACTH) and angiotensin II. Angiotensin II has been shown to meet the criteria of an activator of the PKC pathway in bovine adrenocortical cells [10], although no direct link has yet been established between PKC activation and the biochemical components of the steroidogenic biosynthetic machinery [11]. At the same time, angiotensin II induces release of arachidonic acid by adrenocortical cells, thus suggesting that a phospholipase A_2 activity is stimulated [12] and pointing to the possible subsequent occurrence of lyso-PAF as an available PAF precursor.

The major aim of the present work was to examine whether bovine adrenocortical cells possess the ability to synthesize and release PAF and whether this synthesis was modified on stimulation of these cells by angiotensin II. In addition, PAF binding to adrenocortical cell membranes was characterized. Altogether, the reported findings suggest that PAF may participate in the angiotensin II-induced activation of the differentiated functions of adrenocortical cells, possibly through autocrine or paracrine regulatory loops.

MATERIALS AND METHODS

Chemicals

Acetyl-CoA, phosphatidylserine, phorbol 12-myristate 13-acetate (PMA), diolein, ATP and Trizma base were purchased from Sigma (St. Louis, MO, U.S.A.). Fatty-acid-free BSA was from ICN. Synthetic PAF and lyso-PAF were from Bachem (Bubendorf, Switzerland); [^{14}C]acetyl-CoA (50 mCi/mmol)

Abbreviations used: PAF, platelet-activating factor (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine); lyso-PAF, 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine; PMA, phorbol 12-myristate 13-acetate; PKC, protein kinase C; ACTH, adrenocorticotropin; p[NH]ppA, adenosine 5'-[β -amido]triphosphate.

* To whom correspondence should be addressed.

was from the CEA (Saclay, France); [γ - 32 P]ATP (10 Ci/mmol), 1-*O*-octadecyl-[9,10- 3 H(n)]PAF (60 Ci/mmol) and sodium [3 H]acetate (150 mCi/mmol) were from New England Nuclear, Paris, France. Silica gel 60, chloroform and methanol were purchased from Merck (Darmstadt, Germany).

Cell membrane preparation

Bovine adrenal glands were obtained from the local slaughterhouse. After demedullation, the cortical zone was minced and homogenized in 20 mM-Tris/HCl, pH 7.5, containing 0.25 M-sucrose (Tris/sucrose buffer) with 10 strokes of a motor-driven Potter-Elvehjem apparatus. The homogenate was centrifuged at 500 *g* for 10 min and yielded a pellet hereafter referred to as the nuclear fraction. The resulting supernatant was centrifuged at 10000 *g* for 10 min to yield a mitochondrial pellet. The membrane preparation was obtained by spinning the corresponding supernatant for 1 h at 100000 *g*. The final supernatant was collected as the cytosolic fraction and the pellet containing both microsomal and plasma membranes was resuspended in Tris/sucrose buffer at a concentration of 20–30 mg of protein/ml. All preparation steps were performed at 4 °C. Proteins were assayed according to Lowry *et al.* [13].

Assay of lyso-PAF:acetyl-CoA acetyltransferase activity

Acetyltransferase activity was measured by the incorporation of [14 C]acetyl from radiolabelled acetyl-CoA into [14 C]PAF, using lyso-PAF as the substrate. The standard mixture (500 μ l) contained 4.2 mM-Hepes, pH 7.0, 137 mM-NaCl, 2.6 mM-KCl, 0.25 % BSA and 50 μ l of the subcellular preparation examined (50 μ g of protein). The reaction was started by addition of lyso-PAF (40 μ M) and [14 C]acetyl-CoA (100 μ M, 1 μ Ci) and was carried out for 15 min at 37 °C. It was stopped by adding 1.6 ml of chloroform/methanol (1:2, v/v) followed by 0.5 ml of water and 0.5 ml of chloroform according to the Bligh & Dyer procedure [14]. After vigorous stirring, the samples were centrifuged at 500 *g* for 5 min and the lower organic phase containing [14 C]PAF was evaporated under a nitrogen stream. The radioactive lipid products were analysed by t.l.c. on silica gel 60 using chloroform/methanol/acetic acid/water (50:25:8:4, by vol.). [14 C]PAF was identified by its co-migration with authentic PAF added as an internal standard. The corresponding gel area was scraped off the plate and its radioactivity was counted in Aquasol 2 using a Kontron scintillation spectrometer.

Activation of cell membrane acetyltransferase activity by PKC

PKC was purified to homogeneity from adrenocortical cells according to Pelosin *et al.* [15]. Bovine adrenocortical membrane preparations (100 μ g of protein) were preincubated at 30 °C in a final volume of 400 μ l of 10 mM-Tris/HCl buffer, pH 7.5, containing 5 mM-MgCl₂, 750 μ M-CaCl₂, 100 μ M-ATP, 12 μ g of phosphatidylserine/ml and 2 μ g of diolein/ml, previously sonicated. Purified bovine PKC (40 units) was added when indicated. The reaction was stopped by addition of 2 ml of chilled 10 mM-Tris/HCl buffer, pH 7.5, containing 50 mM-NaF. The mixture was then centrifuged at 100000 *g* for 1 h and the pellet was resuspended for assay of acetyltransferase activity.

Protein phosphorylation

The same incubation procedure as above was applied, with the difference that [γ - 32 P]ATP (100 μ M, 1 μ Ci) was introduced. The phosphorylation reaction was stopped by adding concentrated Laemmli [15a] sample buffer. After boiling for 5 min, samples were analysed by SDS/8 % -polyacrylamide-gel electrophoresis, followed by overnight autoradiography on Kodak X-OMAT films.

For quantitative measurements of 32 P incorporation into proteins, samples were treated with 20 % (w/v) trichloroacetic acid and the precipitates were counted for their radioactivity contents.

[3 H]Acetate cell labelling and [3 H]PAF production

The labelling of adrenocortical cells was carried out according to Chap *et al.* [16]. Primary cells culture were washed twice in Krebs-Ringer buffer containing 0.25 % (w/v) BSA, 20 mM-Hepes, pH 7.4, and 0.1 % glucose. The cell layers (average 1.3×10^6 cells) were labelled for 10 min in the presence of 20 μ Ci of [3 H]acetate/ml. The agonist (angiotensin II, PMA, ACTH) was then added at the indicated concentration. The reaction was stopped by transfer at 0 °C and lipid extraction according to the Bligh & Dyer procedure [14]. [3 H]PAF was isolated by t.l.c. analysis and its radioactivity was assayed in a scintillation spectrometer.

[3 H]PAF binding studies

[3 H]PAF binding to adrenocortical preparations was performed using the procedures described in [17,18]. Briefly, 200 μ g of membrane protein was added to each assay. The binding reaction was carried out for various periods of time at 4 °C in a final volume of 200 μ l of 50 mM-Tris/HCl buffer, pH 7.5, containing 10 mM-MgCl₂ and 0.25 % BSA. Non-specific binding was determined by adding a 1000-fold excess of unlabelled PAF. Membrane-bound PAF was isolated by filtration of the mixture through Whatman GF/C filters under vacuum. The filters were washed with 20 ml of buffer and their radioactivity was assayed by scintillation counting in Aquasol 2. Specific PAF binding represented, on average, 30 % of the total bound radioactivity.

RESULTS

Acetyltransferase activity in bovine adrenocortical subcellular preparations

Initial studies were carried out to detect the presence and examine the subcellular distribution of lyso-PAF acetyltransferase activity in bovine adrenocortical cells. Although low specific acetyltransferase activity was detected in mitochondrial and nuclear fractions (40 ± 10 and 35 ± 8 pmol/min per mg of protein respectively) and in the cytosol (10 ± 8 pmol/min per mg), the bulk of the enzyme activity was found associated with the 100000 *g* adrenocortical pellet. Under standard conditions the specific activity found in this bovine adrenocortical membrane preparation was on average about 0.4 nmol/min per mg of protein. This value is about 20-fold lower than that reported in comparable spleen preparations [19], but it is similar to those observed in other tissues such as liver and kidney cortex in which a specific acetyltransferase activity of about 0.2 nmol/min per mg of microsomal protein has been reported [19].

Kinetic parameters of bovine adrenocortical acetyltransferase

To our knowledge, lyso-PAF acetyltransferase activity had never been described in bovine adrenocortical cells; thus characterization and optimization of the enzyme assay appeared to be a prerequisite to the study of its regulation. As shown in Fig. 1(a), the time course of the reaction indicated that PAF synthesis was linear for up to 20 min under our conditions. Increasing the concentration of acetyl-CoA up to 200 μ M (Fig. 1b) resulted in a linear increase in acetyl incorporation into lyso-PAF; however, higher concentrations of the acetyl donor inhibited the reaction. The reaction was linearly dependent upon lyso-PAF concentration, with an optimum at about 30 μ M (Fig. 1c). The reaction was also linear with regard to protein concentration (Fig. 1d), up to about 80 μ g of protein per assay. These data are in accordance

Fig. 1. Kinetic parameters of bovine adrenocortical lyso-PAF acetyltransferase

Subcellular membrane preparations (100000 g pellet) were obtained and lyso-PAF acetyltransferase activity was assayed as described in the Materials and methods section, as a function of time (a), protein concentration (b), and substrate concentrations (c, d). Values are representative of two different preparations assayed in duplicate.

with the major kinetic properties of acetyl-CoA:acetyltransferase activity described in different cell-type preparations by other research groups [19,20].

Phosphorylation of bovine adrenocortical membrane proteins by PKC

Phosphate incorporation from [γ - 32 P]ATP into the 100000 g pellet proteins was examined either in the absence or in the presence of added purified PKC and/or its cofactors. The patterns of protein phosphorylation obtained under these different conditions are illustrated in Fig. 2. Incubation of the bovine adrenocortical preparation with [γ - 32 P]ATP and $MgCl_2$ resulted in the incorporation of radioactivity into several protein bands (lane 2), thus showing that endogenous protein kinase activity was present. Addition of phospholipids and Ca^{2+} slightly stimulated the incorporation of ^{32}P into proteins of about 50, 110 and 130 kDa (lane 3). Addition of purified PKC in the presence of phospholipids and Ca^{2+} markedly enhanced the phosphorylation of several of these substrates, and additional proteins (15, 25, 37, 40, 46 and 70 kDa) were phosphorylated under these conditions (lane 4). Addition of EGTA to chelate Ca^{2+} strongly decreased these PKC-dependent phosphorylations (lane 5), while endogenous protein kinase activities remained detectable. When the PKC inhibitor H-7 was added together with PKC, lipids and Ca^{2+} , a dramatic decrease in the amount of phosphorylated products was observed (lane 6), including substrates phosphorylated in the absence of added PKC.

Effect of PKC, Ca^{2+} and phospholipids on adrenocortical acetyltransferase activity

The addition of ATP/ Mg^{2+} to the membrane preparations did not affect the basal activity of the acetyltransferase (Fig. 3).

Fig. 2. Phosphorylation by PKC of proteins in bovine adrenocortical membrane preparations

Membrane preparations (100 μ g of protein) or purified PKC (40 units) were incubated with [γ - 32 P]ATP and various additives for 5 min at 30 $^{\circ}C$. Proteins were then analysed by SDS/PAGE and incorporated radioactivity was revealed by autoradiography. Lane 1, purified PKC preparation autophosphorylated in the presence of phospholipids and Ca^{2+} ; lane 2, subcellular preparations incubated in the absence of Ca^{2+} and phospholipids; lane 3, same as lane 2, but in the presence of phospholipids (50 μ g/ml) and Ca^{2+} (750 μ M); lane 4, same as in lane 3, plus PKC (40 units) in the incubation; lane 5, the same as lane 4, plus 1 mM-EGTA in the incubation; lane 6, same conditions as lane 4, plus 100 μ M-H-7.

Fig. 3. Lyso-PAF:acetyltransferase activity in bovine adrenocortical preparations on incubation with PKC under various conditions

Adrenocortical membrane preparations were incubated for 5 min at 30 $^{\circ}C$ in the presence of the additives in various combinations, as indicated. The 100000 g pellets were then collected by centrifugation and assayed for their acetyltransferase activity. The data are the means $\pm$ S.D. (vertical bars) of three independent experiments with assays in triplicate, and are expressed with regard to the basal value in the absence of additive, taken as 100 %, phospholipase.

However, when Ca^{2+} and phospholipids were added together with ATP/ Mg^{2+} , a clear increase in PAF synthesis was observed. Additional introduction of purified PKC further enhanced the acetyltransferase activity, up to an average of 240 % of the basal level. When the PKC inhibitor H-7 was introduced, as well as when Ca^{2+} was chelated with EGTA, PAF synthesis in the presence of PKC fell below control levels. The fact that ATP acted as a phosphate donor in a phosphorylation process was

Fig. 4. Simultaneous assay of lyso-PAF:acetyltransferase activity and protein phosphorylation in adrenocortical membrane preparations incubated with PKC

The subcellular preparations were incubated with PKC and ATP, in either the absence (○, □) or the presence (●, ■) of Ca^{2+} and phospholipids. At different time intervals, lyso-PAF:acetyltransferase activity (■, □) was assayed and is expressed as percentage activation with regard to the basal level; ^{32}P incorporation into proteins (●, ○) was determined in an aliquot of the same sample and is expressed as percentage increase with regard to basal values. Values are the means of two independent experiments assayed in duplicate.

supported by the fact that a non-hydrolysable analogue such as adenosine 5'-[$\beta\gamma$ -imido]triphosphate (p[NH]ppA) could not replace ATP in supporting the protein-kinase-mediated acetyltransferase activation.

A detailed time course study of acetyltransferase activation in parallel with that of total protein phosphorylation in the presence of PKC is shown in Fig. 4. Protein phosphorylation assay and acetyltransferase activity measurements were carried out in samples withdrawn at different time intervals from the same incubation mixture. As illustrated in Fig. 4, the activation of the acetyltransferase was maximal after about 3 min of incubation, and protein phosphorylation increased in parallel with the acetyltransferase activity. When Ca^{2+} and phospholipids were omitted in the presence of EGTA, a negligible increase in both

protein phosphorylation and acetyltransferase activity was observed. These data clearly support the view that the last step of PAF biosynthesis in bovine adrenocortical membrane preparations was modulated through a protein phosphorylation reaction. In addition, the participation of endogenous or exogenous PKC in this process was strongly suggested.

PAF production by bovine adrenocortical cells

In order to investigate whether acetyltransferase activation might operate in the hormonally stimulated intact cells, bovine adrenocortical cells were exposed to steroidogenic concentrations of angiotensin II, after labelling with [^3H]acetate. Production of [^3H]PAF was then monitored, and it was revealed that angiotensin II clearly induced an increase in PAF production by adrenocortical cells. As illustrated in Fig. 5, when [^3H]PAF was assayed in the cell pellet and in the medium, it was observed that the bulk of newly synthesized PAF was released into the cell medium. A time course of adrenocortical cell PAF production on stimulation by angiotensin II was examined (Fig. 5). Synthesis reached a maximum at between 10 and 20 min of treatment. PAF release was dependent upon angiotensin II concentrations in the subnanomolar range, whereas doses higher than 100 nM were without effect. This stimulatory effect was mimicked by the active phorbol ester PMA (100 nM), whereas steroidogenic concentrations of ACTH had no effect on adrenocortical cell PAF production (results not shown).

Binding of radiolabelled PAF to bovine adrenocortical cell membranes

Since PAF was released into the stimulated adrenocortical cell medium, it seemed of interest to examine whether these cells may be sensitive to the exogenous phospholipid. Binding experiments using radiolabelled PAF together with increasing amounts of unlabelled ligand were carried out. Binding to intact cell layers yielded high radioactive background levels, resulting in non-reproducible values of displaceable specific binding. We thus turned to the study of [^3H]PAF binding to membrane preparations from adrenocortical cells. As illustrated in Fig. 6, a saturation curve was obtained: when the data were treated according to Scatchard, they yielded a single class of binding sites for which an apparent K_d of 0.5 nM was calculated. The capacity of this binding system was calculated to be 120 fmol/mg of protein. Maximum specific binding was reached after 3 h of incubation at 0 °C and was abolished in the presence of 1 μM unlabelled PAF.

Fig. 5. PAF production by bovine adrenocortical cells stimulated by angiotensin II

Cells were labelled by a 10 min incubation with [^3H]acetate as detailed in the Materials and methods section. The labelled cells were then stimulated with angiotensin II, either at 10^{-8} M for time-course experiments (a), or at increasing concentrations as indicated for 10 min (b). The cell layer and the medium were then separately extracted by the Bligh & Dyer procedure [14]; newly synthesized [^3H]PAF was isolated by t.l.c. and its radioactivity was counted by a scintillation procedure. [^3H]PAF in the extracellular medium (□) and associated with the cell layer (○) was simultaneously assayed during the time course experiment (a). The data are representative of three independent experiments assayed in duplicate.

Fig. 6. PAF binding to bovine adrenocortical membrane preparations

Adrenocortical membrane preparations were incubated for 3 h at 0 °C with different concentrations (1–10 nM) of [³H]PAF, and membrane-bound radioactivity was assayed as described in the Materials and methods section. Non-specific binding was determined in the presence of 1 μ M unlabelled PAF and subtracted from total binding values to obtain a saturation curve. Inset: data were treated according to Scatchard to calculate PAF binding parameters.

DISCUSSION

PAF was first characterized as a lipid mediator in the inflammatory cell response processes [1]. However, a wide significance of this ether lipid in cell physiology is suggested by reports showing that various cell types are able to synthesize PAF [2,21], which has been identified in human urine and amniotic fluid [22]. On the other hand, a large spectrum of biological activities, including, for example, negative effects on cell growth [23], have been reported. The major aim of the present study was to examine whether PAF could represent a component in the regulation of the highly differentiated functions of an endocrine cell system, i.e. steroidogenic bovine adrenocortical cells. The major observations are as follows. (i) Adrenocortical cells possess the acetyltransferase activity responsible for the final step of PAF biosynthesis. (ii) Studies *in vitro* suggest that adrenocortical acetyltransferase activity is increased by a protein phosphorylation process involving PKC. (iii) PAF is released by bovine adrenocortical cells upon stimulation by steroidogenic concentrations of angiotensin II; this effect is mimicked by an active phorbol ester such as PMA. (iv) Adrenocortical cell membrane preparations exhibit high-affinity binding sites for PAF, suggesting that this lipid mediator may play a role in an autocrine or paracrine regulatory loop during steroidogenic activation of these cells.

Lyso-PAF: acetyl-CoA acetyltransferase was for the first time characterized in adrenocortical cells. The bulk of enzyme activity was found associated with the subcellular fraction usually referred to as microsomal, as previously found in several other cell types [19]. The examined kinetic properties of the adrenocortical enzyme were found to be very similar to those reported in other systems, e.g. with regard to their K_m values for acetyl-CoA (about 50 μ M). The adrenocortical acetyltransferase activity thus appeared not to differ from its previously characterized counterparts, e.g. in rat spleen [24], macrophages or neutrophils [25,26]. The specific activity of the adrenocortical enzyme (0.4 nmol/min per mg of protein) is within the range of values found in several other cell types [19].

Using adrenocortical preparations, it was found that acetyltransferase could be activated *in vitro*. ATP was clearly a required cofactor for this activation, which could be induced in the presence of Ca^{2+} and phospholipids such as phosphatidylserine. Maximal activation (2–3-fold) was obtained upon further ad-

dition of a catalytic amount of purified PKC. A parallel study of microsomal protein phosphorylation showed that the preparations contained active endogenous protein kinase activities, including a phospholipid- and Ca^{2+} -sensitive moiety. The level of protein phosphorylation strikingly paralleled the increase in acetyltransferase activity.

These observations suggest that the adrenocortical particulate preparations contained a PKC-like activity which was responsible for the bulk of Ca^{2+} - and phospholipid-stimulated protein phosphorylation. Addition of authentic PKC further enhanced both protein phosphorylation and acetyltransferase activity. In addition, the PKC inhibitor H-7 markedly inhibited both activities. These data clearly support the hypothesis that adrenocortical acetyltransferase activity is regulated either directly or indirectly by a PKC-mediated phosphorylation process. Regulation of the lyso-PAF acetyltransferase by phosphorylation has been documented by other research groups. Indirect evidence has suggested that PKC activates PAF synthesis in neutrophils [27], while Ca^{2+} /calmodulin-dependent phosphorylation was suggested not to be involved [5]. On the other hand, partially purified acetyltransferase from rat spleen was shown to be activated by cyclic AMP-dependent protein kinase *in vitro* [6], as well as by a Ca^{2+} /calmodulin-dependent protein kinase [28]. We have no direct evidence for a PKC-dependent phosphorylation of the acetyltransferase moiety present in our adrenocortical preparations, since no specific tool such as an anti-transferase antibody is to our knowledge available. One may note, however, that no relationship was obvious between the enzyme activity and the intensity of phosphate incorporation into the 30 kDa protein band region (see Fig. 2), which represents the reported size of the acetyltransferase characterized in rat spleen tissue [6]. Specific tools to characterize the enzyme at the molecular level are obviously required for further study. However, to our knowledge, the transferase has not yet been obtained in a homogeneous form (see [6]) and no specific antibody is available.

The involvement of PKC in acetyltransferase activation was further suggested by studies using intact adrenocortical cells. Steroidogenic stimulation of these cells by angiotensin II has been shown to meet all the criteria to induce cellular PKC activation [10,11]. Angiotensin II treatment induced a dose-dependent activation of PAF synthesis. Since the peptide has been previously shown to activate arachidonate release in these adrenocortical cells [12], one might suggest that angiotensin II triggers both a phospholipase A_2 and the lyso-PAF acetyltransferase activations, in line with a co-ordinated metabolic pathway leading to PAF production [29]. This proposal is further supported by the fact that an active phorbol ester such as PMA, which is believed to directly activate PKC in intact cells, mimicked the angiotensin II effect on adrenocortical cell PAF synthesis. On the other hand, in this cell type, optimal concentrations of the other major steroidogenic peptide (i.e. ACTH) did not induce any detectable change in PAF synthesis. By contrast with angiotensin II, ACTH acts through a cyclic AMP-dependent pathway [30]. This suggests that in adrenocortical cells, PAF synthesis activity is not influenced by an intracellular increase in cyclic AMP and corresponding protein kinase A activation. These observations are in line with the existence of two clearly different activation pathways in bovine adrenocortical cells: (i) a cyclic nucleotide/protein kinase A system, under the control of ACTH, and (ii) the angiotensin II pathway, involving lipidic messengers (phosphoinositides, arachidonate, PAF). The fact that both pathways result in a similar biological response (i.e. acute stimulation of corticosteroid production) confers to the bovine adrenocortical cell model a special interest, since it has been shown that the two activation pathways could 'cross-talk' in these cells [31].

Another observation in the present study was that newly synthesized PAF did not accumulate in bovine adrenocortical cells, but was mostly released into the medium within the first 10 min of angiotensin II exposure. Although this has been generally observed with different cell types [3,32] and blood platelets [16], intracellular retention of synthesized PAF has been reported in human neutrophils [33]. The fact that adrenocortical-produced PAF was secreted into the cell medium suggested that the ether lipid may play either an autocrine or a paracrine role in the overall cell response to an agonist such as angiotensin II. This prompted a study of PAF binding to bovine adrenocortical cell membrane preparations. We found that these cells exhibit specific binding of PAF, represented by a single type of detectable site, with an affinity in the range reported in other cell types [34,35]. These observations strongly suggest that released PAF might affect surrounding adrenocortical cells during angiotensin II stimulation. Preliminary experiments showed that addition of exogenous PAF has no direct effect on steroidogenic activity in bovine adrenocortical cells. This is in contrast with a preliminary report describing a steroidogenic effect of PAF on perfused guinea pig glands and dispersed canine adrenocortical cells [9]. Although we have no clear explanation for this discrepancy, it may be that the reported adrenocortical steroidogenic effect of PAF could involve an indirect pathway, with the contribution of other cell types that are present in dispersed cell preparations and of course in perfusion studies [9], whereas our study used homogeneous fasciculata cell populations in culture. It remains to be established whether the ether lipid might participate in a concerted fashion in the array of intracellular and intercellular regulatory messengers, resulting in the overall regulation of adrenocortical cell differentiated functions.

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