Pergamon

0960-0760(94)E0059-T

*J. Steroid Biochem. Molec. Biol.* Vol. 49, No. 4–6, pp. 359–363, 1994
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0960-0760/94 $7.00 + 0.00

# Platelet-activating Factor in Human Endometrium

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**Platelet-activating factor (PAF) is a phospholipid actively produced by human endometrium and deeply involved in the processes of ovoimplantation and labor. We recently found that PAF represents a new autocrine growth factor for a human adenocarcinoma cell line, HEC-1A. Indeed, biologically active PAF is synthesized by HEC-1A cells, under progesterone control. In HEC-1A cells, PAF regulates intracellular calcium concentration ($[Ca^{2+}]$), DNA synthesis and expression of early oncogenes. All these effects are blocked by the receptor antagonist L659,989. However, while nanomolar concentrations of PAF mobilize $[Ca^{2+}]$, only micromolar concentrations affect cell growth, suggesting heterogeneity of PAF receptors or signaling. Two distinct populations of PAF receptors are present in HEC-1A cells, which bind PAF in nanomolar and micromolar concentrations, respectively. Since HEC-1A cells are producing elevated concentrations of PAF and micromolar concentrations of the PAF antagonist L659,989 inhibit cell proliferation, an autocrine role for PAF is suggested in HEC-1A cells.**

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## PAF IN UTERINE PHYSIOLOGY

Platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-sn-glycero-3-phosphocholine) was initially described as a potent activator of platelet aggregation and local mediator of inflammation [1]. Later on PAF has been shown to be involved in a much wider range of pathophysiological events including asthma, endotoxin shock, gastrointestinal ulceration, cardiac analphylaxis and acute lung injury [1].

Several evidences indicate a role for PAF in uterine physiology and especially in the processes of ovoimplantation and pregnancy. Indeed, the uterus appears to be the only animal tissue containing a significant amount of PAF in physiological conditions [2]. Furthermore, PAF is actively synthesized by human endometrial cells in primary culture both in basal conditions and following stimulation with progesterone [3, 4] and specific receptors for PAF are present in rabbit endometrium, peaking during the implantation period [5–7].

*Proceedings of the XVI Meeting of the International Study Group for Steroid Hormones*, Vienna, Austria, 28 Nov.–1 Dec. 1993.
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### *PAF and ovoimplantation*

A defined role for PAF has been demonstrated at the time of implantation, when increasing amounts of the phospholipid are produced by the embryo [8–10] and the preimplantive uterus, particularly by the endometrium [11, 12]. Moreover, the intravenous administration of PAF receptor antagonists dramatically inhibits implantation in the mouse [13], whereas the intrauterine administration of PAF induces a decidua-like reaction in the pseudopregnant rat [14]. O'Neill *et al.* [15] demonstrated that supplementation of "*in vitro*" fertilization medium with nanomolar concentrations of PAF significantly increased the pregnancy rate of women undergoing *in vitro* fertilization. This effect is probably related to PAF-mediated increased viability of embryos cultured *in vitro* [9, 16, 17], although this has been questioned by some authors [18]. In addition, PAF may affect implantation at the endometrial level by acting on specific receptors which mediate a possible PAF-induced local increase of vascular permeability occurrent around the implantation site [13]. Moreover, it has been recently shown that the administration of PAF in combination with sub-effective doses of alphal-recombinant interferon,

a protein with similar structure to the trophoblastic interferon-like protein oTP-1, prolonged the function of the corpus luteum and resulted in maintenance of progesterone secretion in the ewe [19]. Another possible mechanism is suggested by PAF-induced release of prostaglandins from endometrial cells [20, 21].

*PAF and labor*

Evidence suggests a role for PAF in initiation and maintenace of labor. Firstly, PAF has been shown to induce myometrial contraction in several species [22–25]. In myometrial cells, PAF has been shown to induce an increase of phosphatidylinositol hydrolysis [25] as well as an increase in intracellular $Ca^{2+}$ and phosphorylation of myosin light chain [22]. In addition, PAF and PAF-like activity are present in amniotic fluid from women in labor [26, 27], with a 20-fold elevation in patients incurring preterm labor [27], whereas PAF concentrations in the amniotic fluid from women at term but not in labor were undetectable [26, 27]. Furthermore, the activity of PAF acetylhydrolase, the enzyme which degrades PAF to its inactive metabolite lyso-PAF, is high in rabbit serum during the first days of pregnancy and progressively decreases in late pregnancy [28], possibly contributing to an increase in PAF concentrations at the time of parturition. Finally, the administration of a PAF receptor antagonist to pregnant rats significantly increased the duration of parturition from 2- to 5-fold [29], providing additional support to the view of a role for PAF in the events of parturition.

## PAF AS AN AUTOCRINE FACTOR FOR UTERINE ADENOCARCINOMA CELLS

Most recently, data have emerged suggesting a role for PAF in the control of cell proliferation. Indeed, PAF stimulates tyrosine phosphorylation of proteins both in platelets [30] as well as in proliferating cells [31, 32] and increases mRNA transcripts for the early oncogenes c-*fos* and c-*jun* [32–36]. Moreover, a proliferative effect of PAF has been reported for Raji lymphoblasts, a Burkitt lymphoma-derived cell line [37] and for guinea pig bone marrow cells [38]. In addition, recent data demonstrated an antiproliferative effect of several PAF receptor antagonists towards different human cancer cell lines [39, 40], although this effect has not been related to PAF receptors antagonism. All this evidence, along with the data on production and presence of specific receptors for PAF in human endometrial cells, prompted us to investigate the synthesis and the effects of PAF and its third generation receptor antagonist L659,989 in the human endometrial cancer cell line HEC-1A. This cell line was established in 1968 by Kuramoto *et al.* [41] from explants of adenocarcinoma of human endometrium derived from a patient with stage 1A endometrial tumor. We essentially found that HEC-1A cells not

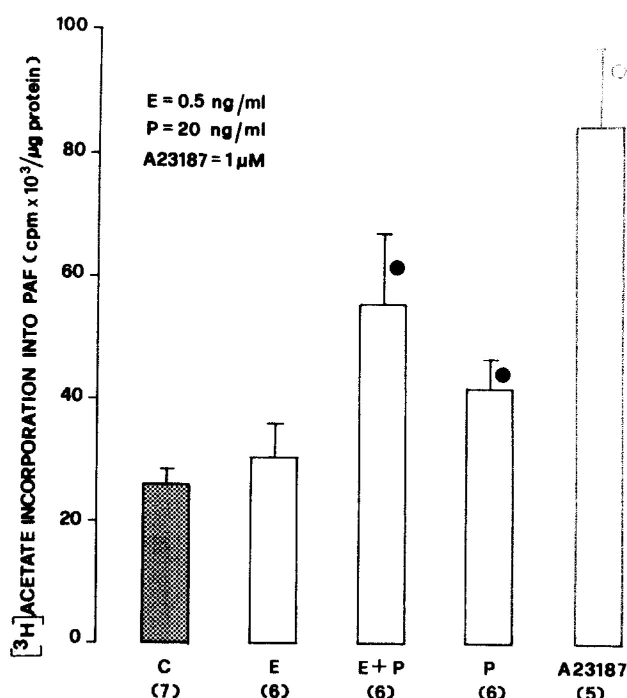


**Fig. 1. Synthesis of PAF ([³H]acetate incorporation into PAF) by HEC-1A cells in basal conditions (C) and following 24 h stimulation with 17-β estradiol (E), 17-β estradiol + progesterone (E + P), progesterone (P) and the calcium inophore A23187. Closed circles = $P < 0.05$; open circles = $P < 0.01$. Number of experiments as in parentheses.**

only synthesize PAF but also contain biologically active receptors which play an important role in the control of cell proliferation.

*Production of PAF from HEC-1A cells*

PAF synthesis was studied by means of [³H]acetate incorporation into PAF in intact cells (which predominantly evaluate the remodeling pathway of PAF synthesis), as well as by qualitative and quantitative gas chromatography-mass spectrometry analysis of PAF-like material extracted from the cells. Results of [³H]acetate incorporation studies revealed a high incorporation of the tritiated molecule into PAF in basal conditions (Fig. 1), suggesting an active production of PAF from HEC-1A cells. PAF synthesis was further stimulated by the calcium ionophore A23187 (1 µM) (Fig. 1), which induced a nearly 3-fold increase in [³H]acetate incorporation into PAF. Figure 1 shows that the production of PAF is not only regulated by

*Table 1. Effects of progesterone and its receptor antagonist RU486 on PAF synthesis expressed as percentage (± SEM) of control values (n = 3)*

| Control | RU486 1 µM | Progesterone 100 nM | Progesterone + RU486 |
|---|---|---|---|
| 100 | 129 ± 12.2 | 173 ± 17.26* | 131 ± 13.3 |

*$P < 0.05$ vs control values.

intracellular calcium concentration but also affected by a 24 h incubation with physiological concentrations of sex steroids. Indeed, progesterone (20 ng/ml) and the combination of progesterone plus estradiol (0.5 ng/ml) induced a significant increase of [$^3$H]acetate incorporation into PAF. Conversely, estradiol alone was without effect. Since the stimulatory effect of progesterone on PAF synthesis was counteracted by the concomitant incubation with the progesterone antagonist RU486 (1 $\mu$M) (Table 1), we suggest the involvement of specific receptors for progesterone. These results are in agreement with those obtained by Alecozay *et al.* [3] in human luteal phase endometrium. Gas chromatography-mass spectrometry analysis of PAF-like material extracted from HEC-1A cells confirmed the synthesis of sustained concentrations of biologically active PAF by the cells [42].

*Biological effects of PAF*

Since numerous studies have indicated that PAF receptor-induced transmembrane signaling mechanisms involve an increase in intracellular calcium concentration ($[Ca^{2+}]_i$), we next studied the effect of PAF on $[Ca^{2+}]_i$ in HEC-1A cells previously loaded with the fluorescent dye fura 2. Nanomolar concentrations of PAF induced a biphasic increase in $[Ca^{2+}]_i$: an initial transient was followed by a sustained phase, which was blunted in the absence of extracellular calcium [42]. Hence, we suggested that PAF induced a dual effect in HEC-1A cells: mobilizing $Ca^{2+}$ from intracellular stores and opening a transmembrane calcium channel. Both effects are completely inhibited by nanomolar concentrations of the PAF receptor antagonist L659,989, indicating the involvement in a specific PAF receptor [42].

We also found that PAF, in micromolar concentrations, induced an increase of mRNA steady-state level for the early oncogene c-*fos*. This effect was time- and dose-dependent and again abolished by concomitant incubation with the antagonist L659,989 [42]. We therefore investigated the effect of increasing concentrations of PAF, L659,989 and the combination of equimolar concentrations of the two agents on [$^3$H]thymidine incorporation in HEC-1A cells. PAF induced a dose-dependent increase ($EC_{50} = 0.7 \pm 0.2\ \mu$M) in DNA synthesis, whereas L659,989 in equimolar concentrations counteracted this effect. Furthermore L659,989 per se dose-dependently inhibited thymidine uptake ($IC_{50} = 2.17 \pm 0.7\ \mu$M). At a concentration of 32 $\mu$M, L659,989 almost completely inhibited DNA synthesis and greatly affected cell proliferation. This effect was apparently reversible, since removing L659,989 by extensive washing completely rescued the inhibitory effect [42]. In aphidicolin-synchronized HEC-1A cells micromolar concentrations of the PAF antagonist L659,989 caused a partial accumulation of cell nuclei in the $G_2$/M phase of the cell cycle, as evaluated by flow cytometry [42]. In order to further evaluate whether the effect of the PAF antagonist was due to a cytotoxic effect, we measured lactate dehydrogenase (LDH) cell content after 24 h of treatment. LDH activity was not different in the treated samples when compared to controls, suggesting that L659,989 was not cytotoxic for the cells. As a control, we evaluated the effect of PAF and L659,989 on thymidine incorporation in the uterine leiomyosarcoma cell line SK-UT-1. Neither PAF nor L659,989 had any effect on cell growth in this cell line, suggesting a specific effect of PAF and its antagonist in the HEC-1A cell line [42].

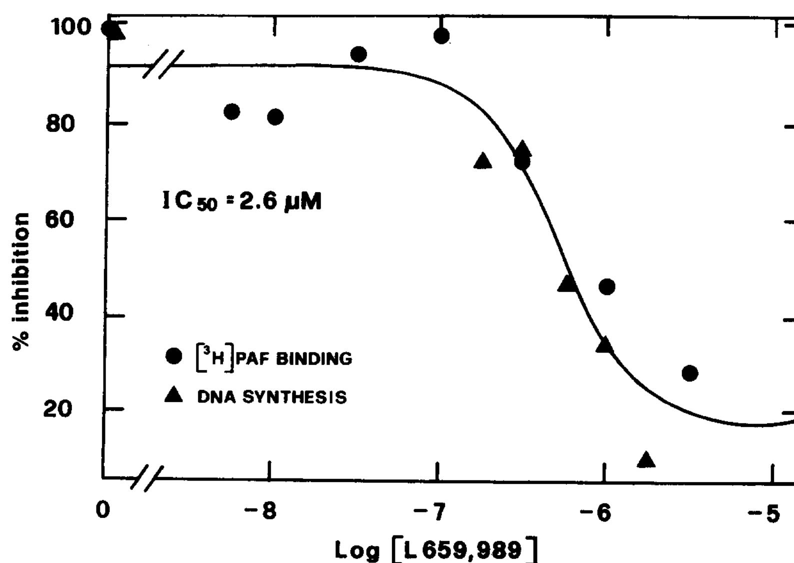


**Fig. 2. Correlation between the concentrations that cause inhibition of either DNA synthesis (closed triangles) or [$^3$H]PAF binding (closed circles). Results from a typical experiment are shown.**

*Receptors for PAF*

We demonstrated the presence of a heterogeneous population of PAF receptors in HEC-1A cells. The first site binds with high affinity but low capacity [$^3$H]PAF, while the second site shows low affinity but higher capacity. Figure 2 shows a typical competition experiment for [$^3$H]PAF binding to the low affinity site, using L659,989 as competitor. In the same figure the effect of the PAF antagonist on DNA synthesis is shown. The two curves could be fit with one equation, strongly indicating a close correlation between the inhibitory concentrations of L659,989 for binding and DNA synthesis.

## CONCLUSIONS

Our results indicate that endometrial adenocarcinoma cells in culture synthesize large amounts of PAF and retain the steroid control on the synthesis of this phospholipid as previously reported in normal endometrial cells [3, 4]. However, the role of PAF appears to be different in normal and cancer cells: modulation of ovoimplantation in physiological conditions, and autocrine regulation of cell growth in the cancer cell line. We found that PAF affects several intracellular pathways in HEC-1A cells, involving the mobilization of intracellular $Ca^{2+}$, the expression of protooncogenes and DNA synthesis. All these effects were counteracted by the specific antagonist L659,989. However, while the effect on $Ca^{2+}$ mobilization were observed at nanomolar concentrations, the effects on cell proliferation were just observed at micromolar concentrations. It is likely that these two distinct effects might be mediated by the two distinct PAF isoreceptors identified in HEC-1A cells. Hence, we hypothesize that the sustained amounts of PAF produced by HEC-1A cells could activate both high and low affinity receptors thus regulating cell proliferation in an autocrine manner.

*Acknowledgements*—This paper was supported by Consiglio Nazionale delle Ricerche (Rome, Italy-Progetto ACRO, contratto No. 92.02266), by Associazione Italiana Ricerca sul Cancro (AIRC, Milano, Italy) and by the AFRCN, Florence, Italy. Dr Giovanna Finetti is a fellowship recipient of the AIRC, Milano, Italy.

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