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Platelet-Activating Factor Contracts Human Myometrium *in Vitro* (42435)

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Abstract. The myometrial contractile responses to synthetic 1-0-octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (platelet-activating factor, PAF) and to oxytocin were evaluated *in vitro* on uterine (lower segment) strips obtained from pregnant women at term (39th week), undergoing elective cesarean section. Contractility was measured isometrically in an isolated organ bath using a superfusion technique. PAF in a concentration range between 5 and 100 n*M* as well as oxytocin (0.1–10 mU/ml) induced a dose-dependent contraction which could be categorized in two patterns, depending on whether spontaneous activity was present. In resting strips, oxytocin induced a prompt (0.5–1 min) development of active tension, followed by a prolonged (6–18 min), slow contraction and a final relaxation. However, at variance with oxytocin, PAF-induced contractions were rhythmic (3–8/hr), and characterized by a prompt (0.5–2 min) development of tension, followed by a brief (0.5–2 min) plateau, and a final, rapid relaxation. In spontaneously active strips, both stimuli induced a marked potentiation of the contractile activity. PAF response was dependent on both cyclooxygenase- and lipoxygenase-derived products as inferred from the abrogating effects of indomethacin and FPL 55712. A receptor-mediated mechanism of action was inferred from the occurrence of specific desensitization to PAF (but not to oxytocin), and from the blocking effect of CV 3988, a specific PAF receptor antagonist. The present study indicates that PAF stimulates the contraction of human myometrium *in vitro* and suggests that this mediator may have a role in labor.

Platelet-activating factor (PAF), structurally identified as a 1-0-hexadecyl/octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine (1, 2), belongs to a new class of lipid mediators of inflammation (3). PAF originates from several tissues (kidney, heart) (4, 5) and individual cell types, such as monocytes–macrophages (6, 7), neutrophils (6, 8), basophils (6), platelets (9), large granular lymphocytes (10), and endothelial cells (11, 12), when appropriately stimulated. PAF has a broad range of biological activities. Besides platelet activation, PAF induces the contraction of visceral smooth muscle (13, 14), a negative inotropic effect on guinea pig (15) and human (16) papillary muscle, the aggregation, chemotaxis, and granule secretion from neutrophils and monocytes, and the increase in vascular permeability (as reviewed in Ref. (17)).

Recently, PAF has been detected in the amniotic fluid after labor (18, 19) and shown to be synthetized *in vitro* by amnion tissue stimulated with calcium ionophore A23187 (18). Furthermore, addition of PAF to isolated amnion tissue promoted increased synthesis of prostaglandin E_2 (PGE_2) in the incubation medium (18).

Prostanoids induce uterine contraction (20). During labor, the conversion of arachidonic acid into biologically active metabolites within the uterus leads to the formation of compounds such as PGE_2, $PGF_{2\alpha}$, PGI_2, thromboxane A_2, hydroxy and hydroperoxy intermediates, as well as leukotrienes, all of which play a complex, regulatory role on the contractility of the pregnant uterus before and during labor (20–27). However, no information so far exists as to whether PAF induces the contraction of the pregnant human uterus.

In this study, we evaluated the contractile response to PAF of myometrial strips obtained from the lower segment of human pregnant uteri and maintained in an isolated organ bath by a superfusion technique.

Materials and Methods. *Stimuli.* Synthetic 1-0-octadecyl-2-acetyl-sn-glyceryl-3-phos-

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phorylcholine (PAF) (Bachem Feinchemikalien AG, Switzerland) was first chromatographed by thin-layer chromatography (TLC) on precoated silica gel plates (60F254, Merck, Darmstadt, FRG) with the use of the highly polar solvent system (methanol:water, 2:1, vol:vol) (28). In this system, a single spot was obtained with an Rf of 0.5. The PAF-containing region was scraped and extracted with a mixture of chloroform:methanol:water (1:2:0.8, vol:vol) which, after centrifugation for the removal of silica gel, was allowed to phase by addition of 1 vol of chloroform and 1 vol of water (29). The PAF-containing chloroform phase was rechromatographed on precoated silica gel plates with the use of chloroform:methanol:water (65:35:6, vol:vol) as the solvent system (29). In this condition, PAF migrated with an Rf value of 0.22 (29). The minimal dose of the lipid material migrating with

lets preincubated with the creatine phosphate–creatine phosphokinase enzymatic system (Sigma Chemical Co., St. Louis, Mo.) (312.5 and 152.5 μg/ml of creatine phosphate and creatine phosphokinase, respectively) and indomethacin (Sigma, 50 μM) was 0.05 nM (28). Oxytocin (Synthocinon (R), Sandoz, Basel, CH) was used as reference substance for myometrial contractility.

Studies on pregnant uterus contractility. Myometrial strips from the lower uterine segment were obtained from nine pregnant (39th week) women (age range, 24–38 years) undergoing elective cesarean section. Patients with anterior wall placental insertion were excluded from this study. None of the patients showed evidence of labor. Immediately after surgical excision, muscle specimens (approximately 0.5 × 0.5 × 1 cm) were placed in 20 ml of chilled (4°C), oxygenated standard Tyrode's solution, containing (in mM/liter) NaCl, 137; Na_2HPO_4, 1.8; $MgCl_2$, 0.5; $CaCl_2$, 2.0; KCl 4.0; glucose 5.5; Hepes, 5, pH 7.4 (16), and brought to the laboratory. The specimens were then dissected free of connective tissue, and cut in 1-mm thick strips along the plane of fibers by means of a stereomicroscope equipped with transluminant light. All specimens were mounted immediately in a 5-ml organ chamber and superfused with oxygenated Tyrode's solution warmed at 37°C at a constant flow rate of 2 ml/min. Specimens were connected to a force transducer (mechanoelectronic transducer tube RCA 5734). The contractile activity was recorded isometrically and the emanating signals were amplified with a Tektronix AM 502 differential amplifier, recorded on a magnetic tape by a Hewlett–Packard 3964 A, visualized on a Tektronix 5103 N oscilloscope, and reproduced by a Hewlett–Packard 7015 B X-Y recorder (16). Specimens were allowed to equilibrate for a period of 120 min under a passive tension of 0.5 g. During the following 30 min, the amplitude of the spontaneous contractile activity was determined and statistically analyzed. Strips that did not respond to oxytocin (0.1–10 mU/ml) were not tested further and were discarded. In some experiments, in order to rule out possible interferences of oxytocin on the subsequent myometrial response induced after PAF challenge, the strips were first challenged with PAF, and then with oxytocin. In order to gain some insight into the mechanism of PAF action on the myometrium, the following substances having selective pharmacological activities were used: indomethacin (Sigma, 10 μM, 30 min before challenge) which inhibits arachidonate cyclooxygenase (30); FPL 55712 (Fisons Pharmaceuticals, London, U.K., 10 μM, 10 min before challenge), a specific leukotriene receptor antagonist (31), and CV 3988 (1 μM, Takeda Chemical Industries, Osaka, Japan, 10 min and immediately before oxytocin and PAF challenge, respectively), a specific PAF receptor antagonist (32). CV 3988 was first dissolved in saline, heated for 5 min at 60°C, and cooled at room temperature. The solution was brought to pH 7.4, and used within 1 hr. In some experiments, the effects of indomethacin and FPL 55712 on the oxytocin response were evaluated in the same experimental conditions as described above.

Results. During equilibration, spontaneous contractions were observed in 69% of the cases. Oxytocin induced a dose-dependent enhancement of contractility that was maximal in both amplitude and duration with 10 mU/ml. However, a lower concentration of oxytocin (1.0 mU/ml) was chosen as the reference dose as it evoked contractions of more reproducible amplitude.

Two patterns of contractile response induced by oxytocin could be distinguished, de-

pending on whether spontaneous activity was present: (i) the prompt development of active tension (in 0.5–1 min), followed by a long-lasting (6–18 min), slow contraction, and a final relaxation in resting strips (Fig. 1A); (ii) a marked potentiation in both amplitude and duration of contractions in spontaneously active strips (data not shown).

PAF stimulated the contraction of myometrial strips in a concentration (5 to 100 n*M*)-dependent manner (Table I), 5 n*M* being the minimal effective dose. The dose of PAF giving 50% of the maximal contraction relative to the response to 1.0 mU/ml of oxytocin was 8 n*M* (ED_{50}). PAF contractile response could be also distinguished in two patterns depending on whether spontaneous activity was present. In resting strips, PAF, but not oxytocin, induced rhythmic (3–8/hr) contractions characterized by a prompt (0.5–2 min) development of tension, followed by a brief (1.5–5 min) plateau, and a final, rapid relaxation (Fig. 1A). PAF evoked a contractile response also when added on myometrial strips, which had not been pretreated with oxytocin (Fig. 1B). Prior addition of PAF (5 n*M*) abolished the contractile response to a subsequent challenge with equimolar concentrations of PAF, but not with oxytocin (1 mU/ml) (Fig. 1B). Nevertheless, the myometrial strips returned to be responsive to PAF within 50–60 min after last challenge. Table I summarizes the effect on PAF response by agents with selective pharmacologic activities. Indomethacin, an inhibitor of cyclooxygenase (30), completely abrogated the contractile response to PAF (Fig. 2A). FPL 55712, a specific leukotriene receptor antagonist (31), fully antagonized the myometrial contractile response to PAF (Fig. 2B). CV 3988, used as a specific PAF receptor antagonist (32), completely abrogated PAF-induced myometrial contractions (10 m*M*), but did not influence the response to oxytocin (Fig. 2C). Similarly, oxytocin-induced myometrial contractions were unaffected by both indomethacin and FPL 55712: 97.6 ± 16.2 and 94.6 ± 18.2, (three experiments), respectively, as mean percentage ± 1 SD of contractility (in respect to the response of untreated strips challenged with 1.0 mU/ml of oxytocin, taken as 100%).

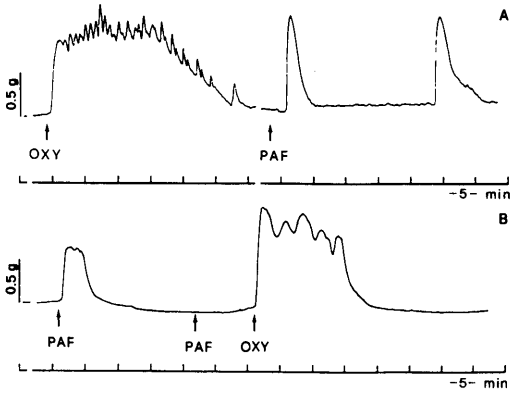

FIG. 1. Typical contractile responses to (A) oxytocin (OXY, 1.0 mU/ml), and PAF (10 n*M*) in resting human myometrial strip; (B) subsequent myometrial response to PAF (5 n*M*) and OXY (1.0 mU/ml) after prior challenge with an equimolar PAF concentration. Each tracing is representative of three to six experiments performed in the same experimental conditions (see Materials and Methods). Vertical calibration = 0.5 g. Horizontal calibration = 5 min.

TABLE I: EFFECTS OF PHARMACOLOGICAL AGENTS ON PAF-INDUCED CONTRACTILE RESPONSE OF MYOMETRIAL STRIPS

	PAF concentration (n*M*)		
Antagonist	5	10	100
Untreated	33.4 ± 8.6 (7)	113.5 ± 11.8 (5)	118.7 ± 13.4 (4)
Indomethacin (10 μ*M*)	ND	0 (3)	7.8 ± 4.0 (4)
FPL 55712 (10 μ*M*)	ND	0 (3)	8.7 ± 2.4 (4)
CF 3988 (1 μ*M*)	ND	0 (4)	7.5 ± 3.6 (4)

Note. PAF-induced contraction (expressed as the mean ± 1 SD of the percentage of contractility evoked by 1.0 mU/ml oxytocin, assumed as 100%) in human myometrial strips untreated or pretreated with various antagonists. The numbers of experiments are given in parentheses. ND not done.

Discussion. The present study provides the first demonstration that PAF evokes a contractile response of myometrial strips obtained from human pregnant uterus. *In vitro* investigations on myometrial contractility must take into account the presence or absence of

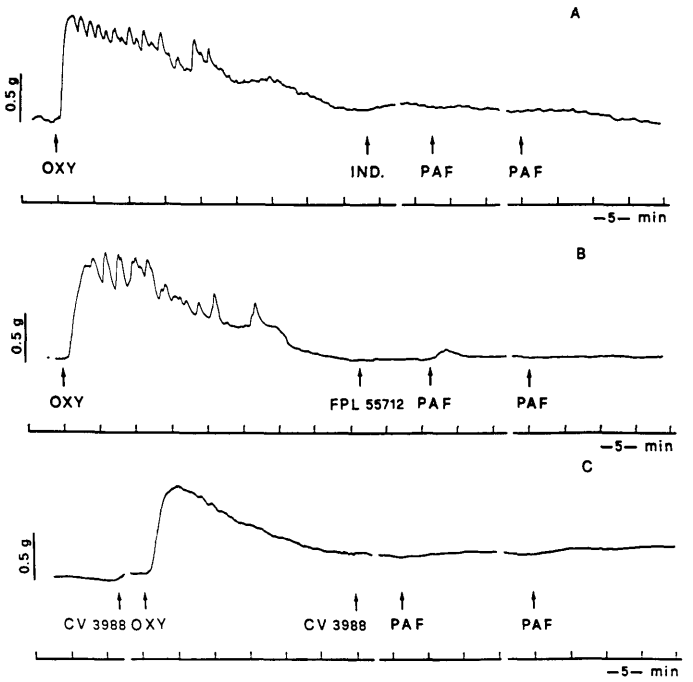

FIG. 2. Typical responses to (A) OXY (1.0 mU/ml), and PAF (from left to right: first challenge, 10 n*M*; second challenge, 100 n*M*), after pretreatment with indomethacin (IND., 10 μM), 30 min before first challenge with PAF; (B) OXY, and PAF (same doses as in A) after pretreatment with FPL 55712 (10 μM), 10 min before first challenge; (C) OXY, and PAF (same doses as in A) after pretreatment with CV 3988 (1 μM) 10 min before OXY and 10 min before the first challenge with PAF. Each tracing is representative of five to seven experiments performed in the same experimental conditions (see Materials and Methods). Vertical calibration = 0.5 g. Horizontal calibration = 5 min.

labor, and the anatomical site of the specimen, as important variables influencing the results (21–23). In this study, we selected pregnant women at term without signs of labor. Furthermore, all specimens were obtained only from the lower uterine segment.

PAF enhanced the amplitude of spontaneous contractions. Furthermore, in resting myometrial strips that had been pretreated with oxytocin, PAF induced rhythmic contractions, characterized by a prompt development of tension followed by a short plateau, finally leading to a rapid relaxation. Finally, PAF evoked a contractile response also in strips which had not been pretreated with oxytocin. On the whole, these results are in agreement with those of Nishihira *et al.* (19), who showed a contractile response of rat myometrial strips to PAF.

The study with pharmacological agents having selective biochemical effects provided some insight into the mechanisms of PAF action on the myometrial strips. PAF response was dependent upon both cyclooxygenase- and lipoxygenase-derived metabolites as indicated by the abrogating effects of indomethacin and FPL 55712. The dependence of PAF-induced contractile response of the myometrium on arachidonic acid-derived metabolites is worth note. In other biological systems, PAF action is independent from cyclooxygenase-derived metabolites as shown for the contractile response of rabbit lung (33), and guinea pig ileum (13), as well as for the activation of rabbit and human platelets (at low concentrations of PAF) (34, 17). On the contrary, on human papillary muscle (16) cyclooxygenase-derived metabolites mediate the mechanical and electrical alterations induced by PAF. Thus, it may be suggested that during labor, PAF, rather than acting directly on myometrium, triggers the synthesis of endogenous prostaglandins not

only by the amnion (18), but also by the myometrium. As inferred from the specific desensitization to PAF and from the inhibitory effect of a PAF receptor antagonist, CV 3988 (32), it appeared that PAF may act through the interaction with specific receptors different from those of oxytocin.

These data may allow some speculations. Prostaglandins and leukotrienes have an established role as uterine contraction-promoting substances (20). Moreover, a marked increase in their levels has been noted after the onset of labor (20). In view of the recent demonstration that PAF is detectable in fetal fluids (urine, tracheal fluids) (35) and, in increased amounts in the amniotic fluid after labor (18, 19), this mediator might stimulate specific receptors present or newly formed on myometrial cells during pregnancy, leading to enhanced synthesis of prostaglandins which may in turn promote uterine contractions.

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