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Increased sensitivity of rat myometrium to the contractile effect of platelet activating factor before delivery

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1 The contractile effects of platelet activating factor (PAF) were compared in the myometrium isolated from non-pregnant and pregnant rats.

2 In the non-pregnant myometrium, PAF, at a concentration of 0.1 μM, did not change muscle tension and induced only a small transient contraction at 10 μM.

3 The contractile responses to PAF increased with the progress of gestation. In the late pregnant myometrium (21 day after gestation), PAF (0.1 nM–10 μM) induced large and relatively sustained contractions. The threshold concentration of PAF was decreased by approximately 10,000 times and the maximum contraction was increased 5 times by day 21 of gestation.

4 PAF (10 μM) increased the cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and muscle contraction to levels higher than those induced by high K^+ in the pregnant rat myometrium (day 21). Verapamil (10 μM), a voltage-dependent Ca^{2+} channel blocker, decreased the stimulated $[Ca^{2+}]_i$ and muscle tension to 49.6% and 22.7%, respectively, while the same concentration of verapamil completely inhibited the high K^+-induced responses.

5 PAF (10 μM) induced a transient increase in $[Ca^{2+}]_i$ with no contraction in the absence of external Ca^{2+} in the pregnant myometrium (day 21).

6 These results suggest that PAF induces contraction in rat myometrium by increasing Ca^{2+} influx. Although PAF released Ca^{2+} from stored sites, this Ca^{2+} does not seem to contribute to the PAF-induced contraction. Our major finding is that the sensitivity of the myometrium to PAF increased after gestation and that this may play a role in delivery.

Keywords: Platelet activating factor; uterine smooth muscle; sensitivity; cytoplasmic Ca^{2+} level

Introduction

Platelet activating factor (PAF) exerts a significant role in physiological and pathophysiological conditions, including platelet aggregation and bronchial asthma (Prescott *et al.*, 1990). It is also well known that PAF induces contraction in the smooth muscle of intestine, stomach and uterus (Findlay *et al.*, 1981; Levy, 1987; Nishihira *et al.*, 1994). Since PAF was detected in amniotic fluid (Hoffman *et al.*, 1990) and since the activity of PAF acetylhydrolase in plasma decreased during late gestation (Maki *et al.*, 1988), it has been suggested that PAF contributes to the progress of gestation. PAF has been reported to increase $[Ca^{2+}]_i$ and myosin light chain phosphorylation in human cultured myometrial cells (Zhu *et al.*, 1992). However, to our knowledge, little is known about the effects of PAF on contraction and $[Ca^{2+}]_i$ in intact uterine smooth muscle. In the present study, we compared the effects of PAF on $[Ca^{2+}]_i$ and the contractile response in uterine smooth muscle strips isolated from non-pregnant and pregnant rats.

Methods

Female Wistar rats (200–250 g) were used for this study. Vaginal smears were taken and the pro-oestrus rats were mated with male rats overnight. The day of gestation when sperm were observed in the vaginal lavage was defined as day 0 of gestation. The normal length of gestation in the colony of rats was 21 days. Uteri of pregnant rats were removed on the appropriate day of gestation. Myometrium, isolated from rats in oestrus, was used as the non-pregnant myometrium. Rats were stunned and bled and a strip of uterine muscle (1–2 mm wide and 7–8 mm in length) was isolated from the middle of each horn in the longitudinal direction.

Each strip was attached to a holder under a resting tension of 10 mN. After equilibration for 20 min in a physiological salt solution (PSS), each strip was repeatedly exposed to 40 mM KCl solution until responses became stable. The PSS contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8; glucose 5.5 and ethylenediaminetetraacetic acid (EDTA) 0.01. The high K^+ solution was prepared by replacing NaCl with equimolar KCl. These solutions were saturated with a 95% O_2 and 5% CO_2 mixture at 37°C and pH 7.4. Muscle contraction was recorded isometrically with a force-displacement transducer (Model TB611T, Nihon Kohden, Tokyo, Japan) connected to a Model 3134 strain amplifier and Model 3056 ink-writing recorder (Yokogawa, Tokyo, Japan).

$[Ca^{2+}]_i$ was measured as described by Ozaki *et al.* (1987) and Sato *et al.* (1988) with a fluorescent Ca^{2+} indicator, fura-PE3. The use of fura-PE3 enabled us to measure $[Ca^{2+}]_i$ for several hours without a significant decline of fluorescence. Muscle strips were treated with acetoxymethyl ester of fura-PE3 (fura-PE3/AM, 5 μM) for 4–5 h at room temperature. A non-cytotoxic detergent, cremophor EL (0.02%), was added to increase the solubility of fura-PE3/AM. After loading, the muscle strip was washed with PSS at 37°C for 20 min to remove uncleaved fura-PE3/AM and was held horizontally in a temperature-controlled, 7 ml organ bath. One end of the muscle strip was connected to a force-displacement transducer to monitor the muscle contraction. The muscle strip was illuminated alternatively (48 Hz) at two excitation wave-lenghts (340 and 380 nm). The intensity of 500 nm fluorescence (F340 and F380) was measured with a fluorimeter (CAF100, JASCO, Tokyo, Japan). The ratio of F340 to F380 (R340/380) was calculated as an indicator of $[Ca^{2+}]_i$. The absolute Ca^{2+} con-

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centration was not calculated in this experiment because the dissociation constant of the fluorescent indicator for Ca^{2+} in cytosol may be different from that obtained *in vitro* (Karaki, 1989). Therefore, the ratio obtained in resting and each stimulant-stimulated muscle was taken as 0 and 100%, respectively.

Drugs used were platelet activating factor C_{18} (1-*O*-octadecyl-2-*O*-acetyl-sn-glycerol-3-phosphocholine, PAF, Cascade Biochem, U.K.), EGTA, EDTA (Dojindo Laboratories, Japan), fura-PE3/AM (Teflabs, U.S.A.) and cremophor EL (Nacalai Tesque, Japan).

Results of the experiments are expressed as mean ± s.e.mean.

Results

Figure 1 shows typical recordings of the effects of PAF on myometria isolated from non-pregnant and pregnant rats. Application of PAF (100 nM) did not change muscle tension in the non-pregnant myometrium ($n=4$). PAF (1 μM), on the other hand, induced small phasic contractions in one of four myometrial strips. PAF, at concentrations of 10 μM and above, induced small phasic contractions and did not produce any tonic contraction ($n=4$, Figure 1a). In the myometrium isolated from rats after 7–8 days pregnancy, a low concentration of PAF (< 10 nM) did not induce any contraction whereas small rhythmic contractions were superimposed on a small sustained contraction at higher concentrations (0.1 or 1 μM). In the myometrium isolated from the day 14–15 pregnant rats, 100 nM PAF induced small repetitive contractions superimposed on the sustained contraction (Figure 1c). In the day 21 pregnant myometrium, 0.1 or 1 nM PAF induced small rhythmic contractions. In this muscle, PAF induced a tonic contraction at 100 nM as shown in Figure 1d. During the sustained increase in muscle tension, transient spontaneous relaxations were observed.

Figure 2 shows the concentration-response relationship for the contractile effects of PAF on myometria at different stages of pregnancy. Since PAF, in many cases, induces rhythmic contractions superimposed on a sustained contraction, either the peak contraction or the steady state contraction was plotted against the PAF-concentration. These data clearly indicate that the sensitivity of the myometrium to PAF contraction increased with time of gestation, and markedly increased towards term.

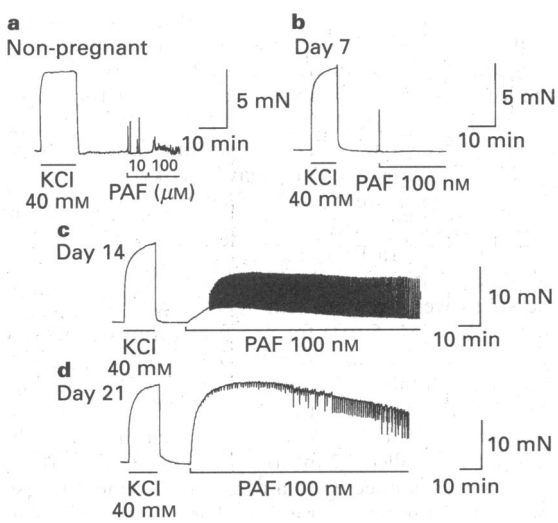

Figure 1 Contractile effects of PAF on rat isolated myometria at different stages of pregnancy (a non-pregnant; b day 7 of gestation; c day 14 of gestation; d day 21 of gestation). After establishing a high K^+ (40 mM)-induced contraction, 100 nM–100 μM PAF was added.

We further examined the effects of PAF on $[Ca^{2+}]_i$ and muscle tension in the day 21 pregnant myometrium. High K^+ (40 mM) induced sustained increases in $[Ca^{2+}]_i$ and muscle tension. Verapamil (10 μM), added after $[Ca^{2+}]_i$ and tension had reached a steady state, completely abolished the high-K^+-induced increase in $[Ca^{2+}]_i$ and the contraction. Application of 10 μM PAF also increased $[Ca^{2+}]_i$ (128.8 ± 11.2% of KCl-response, $n=6$) and muscle tension (152.7 ± 16.1% of KCl-response), effects which were sustained for over 1 h. Addition of 10 μM verapamil inhibited $[Ca^{2+}]_i$ and muscle tension to 49.6 ± 11.3% and 22.7 ± 7.8% of the KCl-response, respectively (Figure 3). Subsequent addition of 4 mM EGTA inhibited $[Ca^{2+}]_i$ and muscle tension below and to the resting levels, respectively.

We also examined the effects of PAF on $[Ca^{2+}]_i$ and muscle tension in a Ca^{2+}-free solution. After establishing high K^+ (40 mM)-induced responses, external Ca^{2+} was removed (with 1.5 mM EGTA) to decrease $[Ca^{2+}]_i$ below the resting level. Application of 10 μM PAF, 1 min after the removal of extracellular Ca^{2+}, induced a transient increase in $[Ca^{2+}]_i$. However, PAF did not increase muscle tension in response to the increase in $[Ca^{2+}]_i$ (Figure 4).

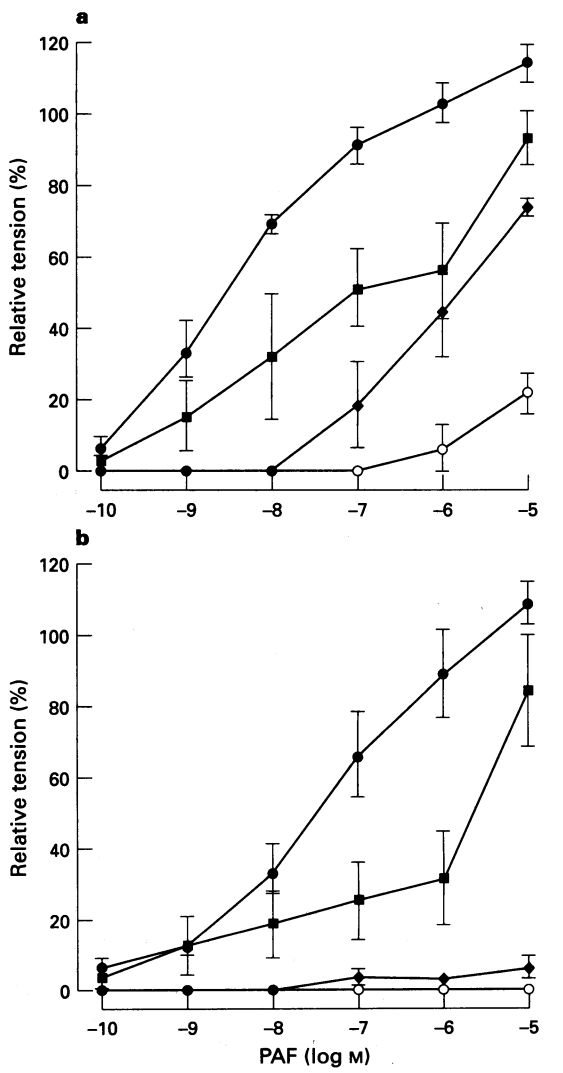

Figure 2 Concentration-response relationships for the contractile effects of PAF on rat myometria at different stages of pregnancy (non-pregnant, ○; day 7–8, ◆; day 14–15, ■; day 21, ●). Since PAF induces rhythmic contractions, either the magnitude of peak response (a) or steady state response (base-line tension) (b) was plotted against the PAF-concentrations; 100% represents the high K^+ (40 mM)-induced contraction obtained just before addition of PAF. Values are expressed as mean ± s.e.mean of 4–13 experiments.

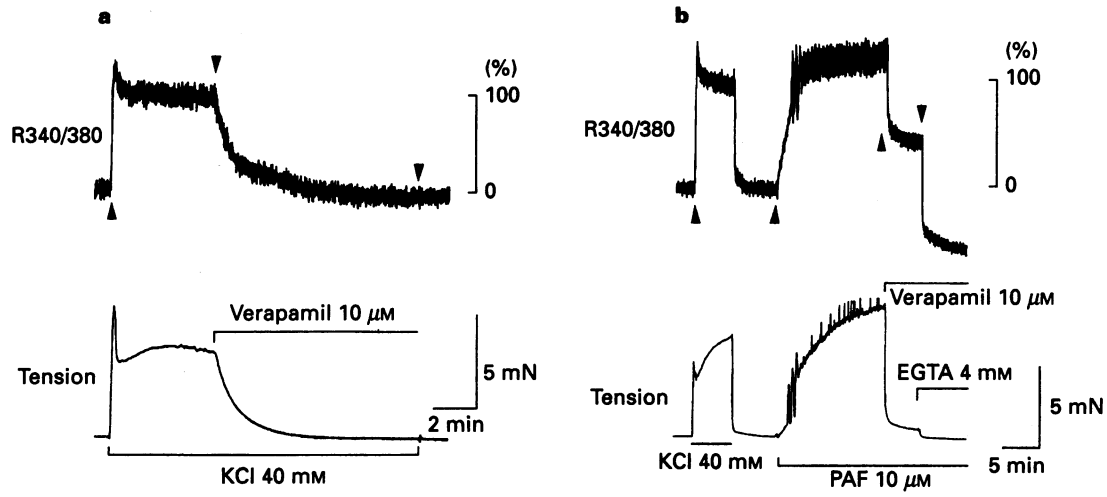

Figure 3 Effect of high K^+ and PAF on $[Ca^{2+}]_i$ (upper trace, indicated by R340/380) and muscle tension (lower trace) in myometria isolated from the day 21 pregnant rat. (a) After the effects of 40 mM KCl were determined, 10 μM verapamil was added. (b) After the response to 10 μM PAF was established, 10 μM verapamil and 4 mM EGTA were added sequentially.

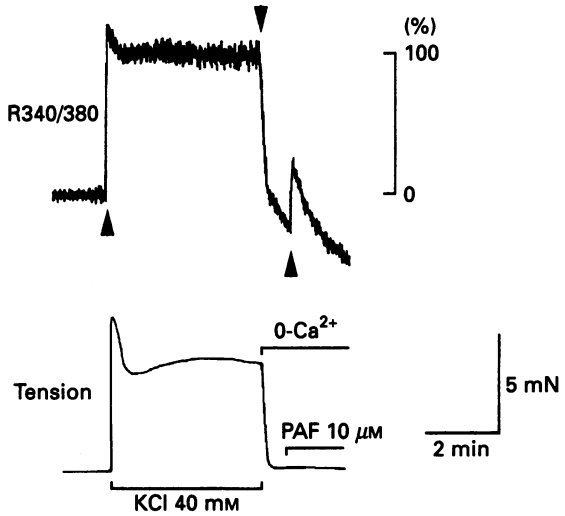

Figure 4 Effect of PAF on $[Ca^{2+}]_i$ (upper trace, indicated by R340/380) and muscle tension (lower trace) in myometrium isolated from day 21 pregnant rat in Ca^{2+}-free solution. After observation of a high K^+ (40 mM)-induced increase in $[Ca^{2+}]_i$, external Ca^{2+} was removed (with 1.5 mM EGTA). One minute later, 10 μM PAF was added.

Discussion

In this study, we found that the myometrial sensitivity to PAF markedly increased during the progress of gestation. Myometria at 7–8 days of gestation exhibited spontaneous rhythmic contractions upon addition of 0.1 μM PAF. In the myometrium near term (day 21), contractions became large and sustained with small rhythmic activities. These results suggest that contractility of myometrium to PAF dramatically increases during late gestation. The increase in sensitivity to PAF may be attributable to an increase in the number of, and/or affinity to, its receptors which may be changed during gestation. It is well known that the number of oxytocin receptors in the myometrium increases at late gestation, an effect which could contribute to a large uterine contraction at delivery (Alexandrova & Soloff, 1980). A decrease in the plasma concentration of acethylhydrolase, an enzyme hydrolysing PAF, during late gestation (Maki *et al.*, 1988) could also enhance the action of PAF on uterine smooth muscle. Furthermore, it has been reported that parturition is delayed by treatment with a PAF antagonist (Zhu *et al.*, 1991). Thus PAF could be another candidate for a physiological regulator during delivery.

The contractile response of smooth muscle is primarily triggered by an increase in $[Ca^{2+}]_i$ (Karaki & Weiss, 1984; Karaki, 1989; Somlyo & Himpens, 1989; Mironneau, 1994). Receptor agonists, such as oxytocin, carbachol and noradrenaline, increase $[Ca^{2+}]_i$ not only through the influx of Ca^{2+} but also by the release of stored Ca^{2+} (Anwer & Sanborn, 1989; Criswell *et al.*, 1994; Szal *et al.*, 1994). It has been also reported that PAF increases $[Ca^{2+}]_i$ and myosin light chain phosphorylation in isolated myometrial cells (Monlar & Hertelandy, 1992; Zhu *et al.*, 1992). In this paper, we confirmed that PAF increases $[Ca^{2+}]_i$ and contraction in intact uterine strips. The increase in $[Ca^{2+}]_i$ due to PAF was partly inhibited by verapamil suggesting that voltage-dependent, L-type Ca^{2+} channels contribute to the increase in $[Ca^{2+}]_i$. Similar results have been obtained in uterine smooth muscle stimulated with endothelin-1 (Sakata & Karaki, 1992). In vascular smooth muscle, it has been suggested that there are Ca^{2+} permeable non-selective cation channels which are insensitive to blockers of voltage-dependent Ca^{2+} channels (Benham & Tsien, 1987; Inoue *et al.*, 1987). Since the non-selective cation channels have been found in the myometrium (Honoré *et al.*, 1989), the verapamil-insensitive increase in $[Ca^{2+}]_i$ may be attributable to Ca^{2+} influx through this type of channel.

In various types of smooth muscles, including vascular, tracheal and gastro-intestinal smooth muscles, receptor agonists induce a larger contraction than high K^+, possibly because the Ca^{2+} sensitivity of contractile elements is increased by receptor stimulation (Karaki, 1989; Somlyo & Himpens, 1989; Ozaki & Karaki, 1993; Sanders & Ozaki, 1994). However, in uterine smooth muscle, PAF and high K^+ increased force to a similar level for a given increase in $[Ca^{2+}]_i$ suggesting that Ca^{2+} sensitivity is not changed by PAF. Similar results have been obtained in rat myometria stimulated by endothelin-1 (Sakata & Karaki, 1992). Thus, Ca^{2+} sensitization may not play a major role in the response of the rat myometrium to agonists.

In smooth muscle, agonists activate phosphatidylinositol (PI) turnover and the generated inositol-1,4,5-trisphosphate releases Ca^{2+} from intracellular Ca^{2+} stores (Somlyo & Somlyo, 1994). Previous work has shown that uterotonic agonists, such as oxytocin, noradrenaline and carbachol, release Ca^{2+} from intracellular Ca^{2+} stores in myometrial cells (Anwer & Sanborn, 1989; Monlar & Hertelandy, 1990; Mironneau, 1994). In the present study, we found that PAF induced a

transient increase in $[Ca^{2+}]_i$-free solution, suggesting that PAF may also activate PI turnover in the myometrium. However, the transient increase in $[Ca^{2+}]_i$ was not accompanied by contraction possibly because the increase in $[Ca^{2+}]_i$ did not reach the threshold for contraction. The threshold $[Ca^{2+}]_i$ for contraction was estimated to be ~50% of that induced by 40 mM KCl because verapamil decreased the PAF-induced increase in $[Ca^{2+}]_i$ to approximately 50% but it decreased the contraction to or near the resting level.

In summary, we showed for the first time that PAF induces a greater contraction in the myometrium at the stage of late pregnancy. The increased sensitivity of PAF may contribute to produce a large contraction of the myometrium during parturition. It was also found that PAF increased $[Ca^{2+}]_i$ by increasing influx of Ca^{2+} through verapamil-sensitive and -insensitive channels. PAF also increased $[Ca^{2+}]_i$ by release of stored Ca^{2+}, although alone the amount of released Ca^{2+} was insufficient to contraction.

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