Platelet-activating factor mobilises intracellular calcium in vascular smooth muscle cells

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The effect of platelet-activating factor (PAF) on polyphosphoinositide metabolism and $^{45}Ca^{2+}$ efflux was examined in a vascular smooth muscle cell line (A_7r_5). PAF stimulated a rapid but transient production of inositol trisphosphate and inositol bisphosphate which, in the presence of lithium, resulted in an accumulation of inositol monophosphate. In addition, PAF induced a rapid efflux of $^{45}Ca^{2+}$ from preloaded cells, an effect which was concentration-dependent. These data suggest that PAF mobilises intracellular Ca^{2+} via the production of inositol trisphosphate.

Ca^{2+} Inositol phosphate Platelet-activating factor (Rat smooth muscle)

1. INTRODUCTION

Platelet-activating factor (PAF, AGEPC, APRL, Paf-acether) is an ether-linked phospholipid implicated in a diverse range of pathological conditions including asthma [1], circulatory disorders [2] and inflammation [3]. PAF was originally described as a potent platelet stimulant inducing shape change, granule release and aggregation at concentration as low as 10^{-12} M [4–6]. However, recent studies indicate that PAF has potent biological activity in a number of other cell types in vitro and in vivo (reviews [3,7]) although little is known about the biochemical basis for such activity. It is, however, recognized that PAF has highly specific effects which are often dependent on Ca^{2+} and can be associated with changes in phospholipids [5,7–9]. Here, we have evaluated the biochemical changes induced by PAF in a smooth muscle cell line orginating from rat aorta.

2. MATERIALS AND METHODS

2.1. *Materials*

[^{3}H]Inositol and $^{45}Ca^{2+}$ were obtained from Amersham International, England. PAF was purchased from Bachem, Bubendorf, Switzerland. PAF antagonist (CV 3988) was a kind gift of Takeda, Japan. All other chemicals used were of the highest grade purity available.

2.2. *Cell culture*

The rat aortic smooth muscle cell line A_7r_5 was obtained from the American Type Tissue Culture Collection, Rockville, MD. The cells were trypsinised and subcultured at a density of 7000 cells/cm^2 in dishes of 16 mm diameter. Experiments were carried out on confluent monolayers of cells between the 7 and 14th day after plating. Measurement of inositol phosphates and $^{45}Ca^{2+}$ efflux was carried out as in [10].

3. RESULTS

In smooth muscle cells which had been prelabelled to equilibrium with [^{3}H]inositol, stimulation with PAF (10^{-7} M) resulted in the formation of $InsP_1$, $InsP_2$ and $InsP_3$. $InsP_3$ production reached a peak level at approx. 30 s and declined to control levels within 5 min (fig.1a). Similarly, production of $InsP_2$ was transient with maximum levels occurring between 1 and 3 min, thereafter declining towards control levels. Formation of $InsP_1$ pro-

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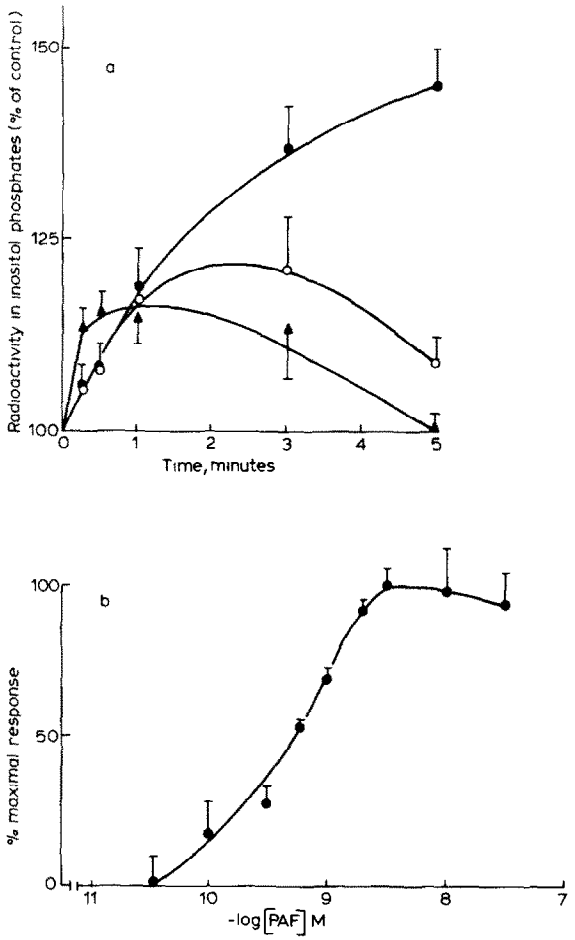

Fig.1. Effect of PAF on inositol lipid metabolism in vascular smooth muscle cells. (a) Kinetics of PAF-mediated $InsP_1$ (●—●), $InsP_2$ (○—○) and $InsP_3$ (▲—▲) production in cells prelabelled with [^{3}H]inositol ($n=7$). (b) Concentration-response relationship of PAF-mediated $InsP_1$ production ($n=4$).

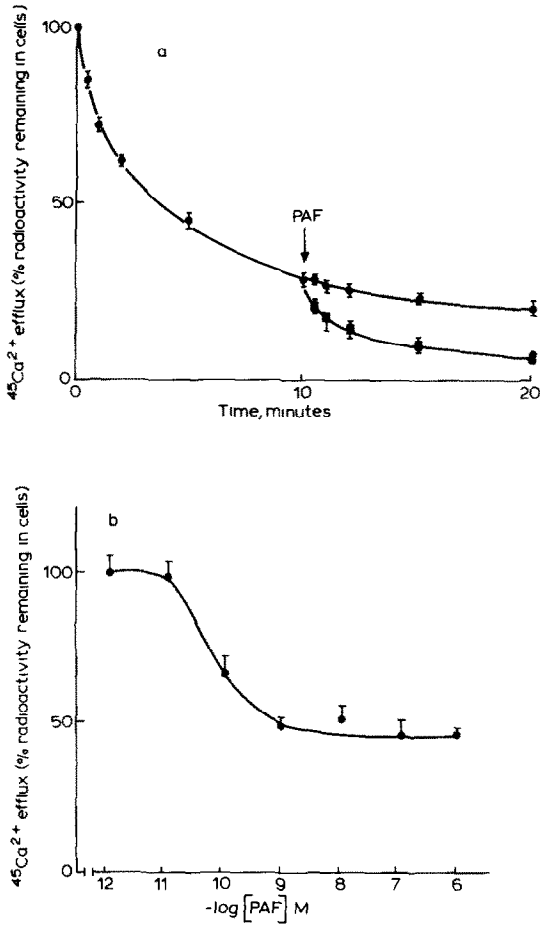

Fig.2. The effect of PAF on $^{45}Ca^{2+}$ efflux in vascular smooth muscle cells. (a) Kinetics of the PAF-stimulated $^{45}Ca^{2+}$ efflux in preloaded cells ($n=3$). (b) Concentration-response curve of PAF-stimulated $^{45}Ca^{2+}$ efflux ($n=5$).

ceeded at a much slower rate than that of $InsP_3$ but continued increasing up to 5 min, the latest time point measured. Accumulation of inositol phosphates as $InsP_1$ following receptor stimulation in the presence of lithium is a sensitive assay of activity of Ca^{2+}-mobilising hormones [11]. Fig.1b shows the concentration dependence of PAF-induced $InsP_1$ accumulation measured at 10 min in the presence of lithium. The concentration of PAF required to provoke half-maximal $InsP_1$ production was 5×10^{-10} M.

Fig.2a shows the kinetics of PAF-induced $^{45}Ca^{2+}$ efflux from preloaded cells. PAF (10^{-7} M) was added after 10 min of efflux in PSS and the $^{45}Ca^{2+}$ remaining in the cells was measured after an additional 10 min. PAF reduced the $^{45}Ca^{2+}$ remaining in the cells to 50% of that in the control, indicating a rapid stimulation of efflux. Using the same protocol, the EC_{50} value for PAF-induced $^{45}Ca^{2+}$ efflux was 4×10^{-11} M, with efflux being essentially complete at 10^{-9} M (fig.2b).

Incubation of smooth muscle cells with the PAF antagonist CV 3988 (10^{-5} M) or indomethacin (10^{-6} M), a cyclooxygenase inhibitor, affected neither $InsP_1$ levels nor basal Ca^{2+} efflux. However, CV 3988 completely abolished those stimulat-

Table 1

Effects of various agents on basal and PAF-stimulated $InsP_1$ accumulation and $^{45}Ca^{2+}$ efflux

	$InsP_1$	$^{45}Ca^{2+}$
Control	100 ± 2	100 ± 3
PAF	223 ± 9*	59 ± 3*
CV 3988 (10^{-5} M)	108 ± 6	98 ± 7
PAF + CV 3988 (10^{-6} M)	190 ± 7*	68 ± 6*
PAF + CV 3988 (10^{-5} M)	101 ± 3	101 ± 4
Indomethacin	97 ± 4	98 ± 8
PAF + indomethacin	221 ± 10*	59 ± 1*
Lyso-PAF (10^{-7} M)	95 ± 7	99 ± 4

The concentrations of PAF used were 10^{-8} M and 10^{-9} M for $InsP_1$ accumulation and $^{45}Ca^{2+}$ efflux, respectively; the concentration of indomethacin used was 100-times that of PAF. Results are presented as % of the control values (mean ± SE, $n = 3$–5). The $^{45}Ca^{2+}$ efflux data are presented as % $^{45}Ca^{2+}$ remaining in the cells. Significance levels, as compared to control values, were assessed by Student's t-test; $*p < 0.001$. CV 3988; *rac*-3-(*N*-*n*-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazolioethyl phosphate

ed by PAF whereas indomethacin was ineffective (table 1).

4. DISCUSSION

Binding sites for PAF have been shown to be present on a number of cell types including platelets [12–14], neutrophils [15,16], guinea pig smooth muscle membranes [12] and lung tissue [17], suggesting that PAF exerts its effects via a specific extracellular receptor. Investigation of the post-receptor events intrinsic to PAF action has been carried out mainly in platelets where PAF can elevate the intracellular calcium level, $[Ca^{2+}]_i$, although it stimulates shape change even when $[Ca^{2+}]_i$ is suppressed [18]. PAF also stimulates a rapid degradation of $PtdInsP_2$ and production of phosphatidic acid [8,19–22]. Such results indicate that PAF induces a phospholipase C-mediated breakdown of $PtdInsP_2$, which is thought to be the initial event in the widely documented transducing mechanism of hormones and neurotransmitters which utilise Ca^{2+} as a second messenger [23,24]. Hydrolysis of $PtdInsP_2$ yields 2 products, $InsP_3$, thought to serve as a second messenger for the mobilization of $[Ca^{2+}]_i$ [25–27] and 1,2-diacylglycerol which activates protein kinase C, which in platelets is considered to be a necessary step for shape change and aggregation [9,28]. In this study we demonstrate that in smooth muscle cells, PAF stimulates the production of $InsP_3$ and subsequent release of $^{45}Ca^{2+}$.

In A_7r_5 cells PAF stimulates production of inositol phosphates with a kinetic pattern similar to those reported in other cultured cells stimulated with various Ca^{2+}-mobilising ligands [10,29]. Production of $InsP_3$ is more rapid than that of $InsP_2$ or $InsP_1$, but also declines rapidly. $InsP_1$ production exhibits a much slower time course but due to the presence of lithium, which inhibits inositol-1-phosphatase, continues over a longer period of time [11]. Thus, as described for other systems [10,30–33], $InsP_3$ derived from $PtdInsP_2$ breakdown is rapidly degraded by phosphomonoesterases to $InsP_1$ via $InsP_2$. The production of $InsP_3$ is sufficiently rapid to account for the equally rapid efflux of Ca^{2+}, indicating that, as previously described in other cell systems, $InsP_3$ could be responsible for intracellular Ca^{2+} release.

The concentration of PAF required to provoke half-maximal accumulation of $InsP_1$ was 5×10^{-10} M whereas half-maximal efflux of Ca^{2+} required a lower concentration of PAF, 4×10^{-11} M. Thus, maximal Ca^{2+} efflux will occur when inositol lipid hydrolysis is only partially activated. This has been observed in other cells [34] and has been explained as a receptor reserve. Under these conditions, any reaction essential to receptor-response coupling, e.g. activation of adenylate cyclase or $PtdInsP_2$ depletion, may only be submaximally activated even though a full physiological effect is observed [35].

A curious feature of PAF antagonists is they are required in high concentrations, up to 1000-fold greater than the PAF concentration. For example, complete inhibition of PAF (3×10^{-8} M)-induced rabbit platelet aggregation by the synthetic PAF antagonist CV 3988 is achieved at 3×10^{-5} M [36]. Similarly in this study, 10^{-5} M CV 3988 was necessary to cause complete inhibition of the response elicited by 10^{-8} M PAF in $InsP_1$ accumulation and Ca^{2+} efflux. Nevertheless, the experimental observations indicate that both PAF-mediated $InsP_1$ production and Ca^{2+} efflux are related events which are the consequence of the interaction of PAF with an extracellular receptor. In

support of this are our findings that lyso-PAF is unable to mimic the action of PAF and indomethacin is unable to inhibit the effects of PAF.

In conclusion, our data support the concept that PAF interacts directly with a specific receptor on the smooth muscle cell. Hwang et al. [12] have shown specific binding sites for PAF on several tissues, including rabbit ileal smooth muscle membranes, with a dissocation constant in the nanomolar range. Our findings support the view that these sites are functional receptors, the stimulation of which results in phospholipase C-mediated degradation of $PtdInsP_2$ and the subsequent production of $InsP_3$ and 1,2-diacylglycerol. $InsP_3$ has been reported to promote the release of Ca^{2+} from an intracellular store, probably the endoplasmic reticulum [25-27]. This effect of PAF appears to be specific as it is blocked by a PAF antagonist, CV 3988. However, this may not be the sole mechanism involved in PAF stimulation. Further studies of the biochemical events in PAF stimulation may facilitate the elucidation of the role of PAF in many diverse pathological conditions.

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