# Acetyl glyceryl ether phosphorylcholine (platelet-activating factor) mediates heightened metabolic activity in macrophages

## Studies on PGE, $TXB_2$ and $O_2^-$ production, spreading, and the influence of calmodulin-inhibitor W-7

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Received 28 June 1983

The phospholipid mediator AGEPC (acetyl glyceryl ether phosphorylcholine) was examined for its effects on guinea pig peritoneal macrophages. At a concentration of $10^{-9}$–$10^{-6}$ M, AGEPC evoked release of prostaglandin E (PGE) and thromboxane $B_2$ ($TXB_2$) from albumin-elicited macrophages. It also triggerd generation of $O_2^-$ by *Corynebacterium parvum*-induced cells. Moreover, it caused augmented spreading of macrophages. The calmodulin antagonist W-7 attenuated AGEPC-mediated $O_2^-$ production and cell spreading whereas prostanoid synthesis was enhanced. These novel actions of AGEPC on the major cellular component of the inflammatory process attest to its role as a potent mediator of immunoinflammatory responses.

*Platelet-activating factor* *Macrophage* *Prostanoid* *$O_2^-$* *Spreading* *Calmodulin antagonist W-7*

## 1. INTRODUCTION

Acetyl glyceryl ether phoshorylcholine (AGEPC, platelet-activating factor) is a phospholipid mediator originating from basophils, platelets, neutrophils, monocytes, and macrophages (mϕ) that causes platelet activation, aggregation and degranulation of neutrophils, hypotension and bronchoconstriction and has been implicated in anaphylactic shock [1,2]. We have recently shown that AGEPC triggers in peritoneal mϕ a burst of luminol-dependent chemiluminescence and release of $H_2O_2$ [3]. To characterize in more detail mϕ activation mediated by AGEPC we examined its effect on the conversion of arachidonic acid (AA) to prostaglandin E (PGE) and thromboxane $B_2$ ($TXB_2$), the release of superoxide anion ($O_2^-$) generated in the oxidative burst, and on the spreading behavior of mϕ, all responses representing sensitive markers of augmented mϕ function [4,5]. Furthermore, in view of the role attributed to calcium fluxes in activation processes of phagocytic cells and their presumed dependence on calmodulin [6–10], we investigated how the calmodulin inhibitor W-7 (*N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide) [11] would affect AGEPC-dependent mϕ responses.

## 2. MATERIALS AND METHODS

C 18 analog of AGEPC (1-*O*-octadecyl-2-acetyl-sn-glycero-3-phosphorylcholine) was purchased from Bachem (Bubendorf), W-7 from Paesel (Frankfurt). Hartley guinea pigs were injected i.p. with human serum albumin (HSA) or *Corynebacterium parvum* (C.p.) 4 or 14 days before sacrifice, respectively [12,13]. Monolayers of mϕ were established as described and consisted of

*Published by Elsevier Science Publishers B.V.*
00145793/83/$ 3.00 

91–95% mϕ as evidenced by non-specific esterase staining and latex phagocytosis, and were free of platelets as revealed by phase contrast microscopy [12,13]. PGE and $TXB_2$ in cell-free culture supernates were determined by radioimmunoassay as in [12,13]. Release of $O_2^-$ was measured by cytochrome *c* reduction [13]. PGE/$TXB_2$ synthesis was studied in HSA-elicited mϕ, $O_2^-$ generation in C.p.-elicited mϕ since these mϕ populations have been shown to be most suitable for the respective studies [12–14]. Spreading of mϕ on surfaces of culture wells (Cluster 6, Costar, Cambridge) was assayed as in [5,15] and results are expressed as % cells spread using criteria outlined in [5,15]. Cell viability was assessed by measuring release of the cytoplasmic enzyme lactate dehydrogenase (LDH) which for concentrations of AGEPC up to $10^{-6}$ M and W-7 up to $3 \times 10^{-5}$ M did not exceed 10% of cellular content and was no different from values obtained in control incubates.

## 3. RESULTS

AGEPC evoked a dose–responsive release of PGE and $TXB_2$ from HSA-elicited mϕ at $10^{-9}$–$10^{-6}$ M which could be completely abolished by addition of the cyclooxygenase inhibitor indomethacin (0.5 μg/ml) (fig.1A). Kinetic analysis revealed that PGE production proceeded at an almost constant rate for 3 h when a plateau was reached (fig.1B). A similar time course was observed for $TXB_2$ liberation (not shown). $O_2^-$ formation by C.p. mϕ was likewise stimulated at $10^{-8}$–$10^{-6}$ M. Kinetics followed the same time course as found for $H_2O_2$ [3] (not shown). Examining its effects on mϕ spreading AGEPC was observed to increase the percentage of cells covering a larger surface on culture wells (table 1). Principally, similar results were obtained in C.p. mϕ except that, due to a higher number of untreated cells spread, increments were smaller (not shown). W-7 added to mϕ at various doses 2 min before challenge with AGEPC, diminished both $O_2^-$ generation and spreading (fig.2). Sensitivity of these stimulating effects of AGEPC toward W-7 was slightly different as is apparent from dose–response curves depicted in fig.2. In contrast, liberation of PGE and $TXB_2$ was not adversely affected by W-7 but rather enhanced in its presence. AGEPC-stimulated PGE synthesis for example, was increased by 17 ± 4/31 ± 7% in the presence of 10/20 μM W-7, respectively, as compared to production induced by AGEPC ($10^{-7}$ M) alone (means ± SD of 4 expt). W-7 effects at these concentrations were non-toxic as indicated by unchanged LDH release from mϕ as compared to controls. Above 25 μM however, W-7 was found to impair viability of mϕ as judged by enhanced leakage of LDH.

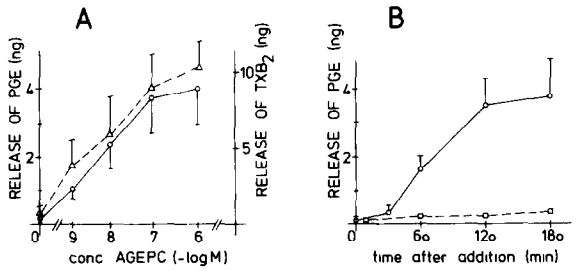


Fig.1. (A) Stimulation of PGE/$TXB_2$ release. HSA-elicited mϕ were challenged with AGEPC. PGE/$TXB_2$ release was determined after 3 h. Results corrected for cell number by gauging DNA content of monolayers [3,12,13] are given per $10^6$ mϕ and represent means ± SD of 5 experiments. Circles: PGE; triangles: $TXB_2$. (B) Kinetics of PGE release. HSA-mϕ were exposed to AGEPC ($10^{-7}$ M), and PGE-release was measured at indicated intervals. Results given per $10^6$ mϕ, means ± SD of 4 experiments with duplicate cultures.

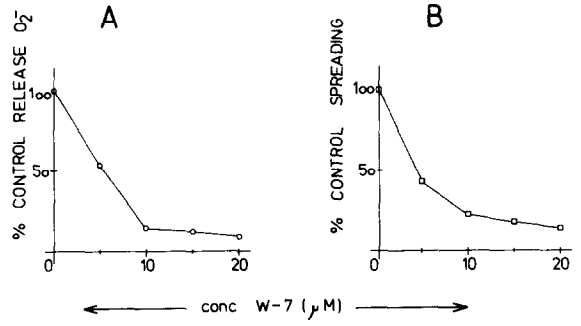


Fig.2. Influence of W-7. 2 min before challenge with AGEPC ($10^{-7}$ M) mϕ were exposed to various doses of W-7. $O_2^-$ release from C.p. mϕ was determined at 30 min, spreading was assessed at 4 h. Results expressed as % of controls exposed to AGEPC ($10^{-7}$ M) only. Means of 5 experiments.

Table 1

Effect of AGEPC on $O_2^-$ production and spreading of mϕ

| [AGEPC] (M) | $O_2^-$ (nmol/$10^6$ mϕ) | % Cells spread |
|---|---|---|
| $10^{-9}$ | 1.6 ± 0.4 | 22 ± 5 |
| $10^{-8}$ | 6.9 ± 1.3 | 39 ± 7 |
| $10^{-7}$ | 13.6 ± 2.4 | 51 ± 9 |
| $10^{-6}$ | 19.9 ± 3.2 | 67 ± 10 |
| Control | 1.5 ± 0.3 | 17 ± 4 |

$O_2^-$ release from C.p.-elicited mϕ was determined 30 min after addition of AGEPC, spreading of HSA-mϕ was assessed 4 h after challenge with AGEPC. Control: mϕ incubated in medium. Means ± SD of 6 expt with triplicate cultures

## 4. DISCUSSION

These findings indicate that AGEPC causes profound changes in the functional status of mϕ. Enhanced metabolic activity of mϕ as evidenced by production of AA cyclooxygenation products PGE and $TXB_2$, $O_2^-$, and augmentation of spreading are novel effects of AGEPC. PGE and $TXB_2$ are known to display phlogistic properties [12,13]. $O_2^-$ and derived reactive oxygen species have been invoked in mϕ-mediated microbial killing, cytotoxicity, destruction of endothelium and tissues [16]. Considering these effects, our results further attest to the emerging stature of AGEPC as a potent mediator of immunoinflammatory responses. Two responses to AGEPC are reduced in magnitude to a variable degree by W-7. Much evidence has been advanced that calcium is pivotal in initiating oxidative burst, phagocytosis and lysosomal enzyme release in neutrophils and mϕ [6–10]. Moreover, the presence of calmodulin in mϕ has recently been demonstrated [9]. Taken together, the observed effects of W-7 on AGEPC-mediated mϕ responses, at concentrations similar to those found to suppress degranulation and $O_2^-$ release from neutrophils [17,18], could be explained by its calmodulin-inhibiting properties although unspecific hydrophobic interactions cannot be totally excluded [19]. Assuming that W-7 acts by virtue of calmodulin antagonism, several sites of action are conceivable. With regard to prostanoid synthesis phospholipase $A_2$ and C have been invoked as rate-limiting steps in AA metabolism of phagocytes although recent evidence suggests that phospholipase C and phosphatidylinositol breakdown are more important in providing free AA for conversion to eicosanoids [20–23]. Enhanced production of PGE and $TXB_2$ in response to the joint action of AGEPC and W-7 is consistent with the demonstration that phenothiazine calmodulin-inhibitors liberate AA from mϕ possibly through activation of diacylglycerol lipase [23]. In platelets phospholipase C-mediated events have been found to be stimulated by calmodulin-antagonists [24]. Diminution of $O_2^-$ generation by W-7 is in accord with findings in neutrophils [17,18] and presumably the result of interacting with NADPH-oxidase. Cell-spreading is a calcium-dependent process [25], and trifluoperazine has been demonstrated to reduce spreading of cultured epithelial cells and fibroblasts [26]. In conclusion, AGEPC causes mϕ activation and production of proinflammatory compounds. These findings demonstrate important interactions between the phospholipid mediator AGEPC and the major cellular component of the inflammatory process.

## ACKNOWLEDGEMENTS

I am most grateful to Professor H.J. Freund and Professor K.V. Toyka for their continuous support. Thanks to Professor K.V. Toyka for reviewing the manuscript. Supported by the Ministerium für Wissenschaft und Forschung, NRW, and the Deutsche Forschungsgemeinschaft SFB 200 B 5.

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