The fluorescence response of chlortetracycline-loaded human neutrophils is modulated by prostaglandin E_1, but not by cyclic nucleotides

James E. Smolen

Division of Pediatric Hematology/Oncology, University of Michigan Medical School, F6515 Mott Children's Hospital, Ann Arbor, MI 48109, USA

Received 12 August 1983

Human neutrophils preloaded with chlortetracycline, commonly used as a probe of membrane-bound calcium, demonstrate a prompt decrease in fluorescence when exposed to surface stimuli such as the chemotactic peptide fMet–Leu–Phe. The fluorescence response was highly sensitive to preincubation with prostaglandin E_1. This effect was apparently not due to elevated levels of cAMP since exogenous dibutyryl-cAMP did not alter the chlortetracycline fluorescence response to fMet–Leu–Phe. This is one of the few instances of prostaglandin E_1 affecting neutrophils at physiologic concentrations, dissociated from changes in cellular cyclic nucleotide levels.

Fluorescence *Chlortetracycline* *Neutrophil* *Prostaglandin E_1* *Cyclic nucleotide*

1. INTRODUCTION

Exposure of human neutrophils to any one of a variety of stimuli provokes a cascade of measurable responses, such as superoxide anion generation and lysosomal enzyme release, both of which are delayed relative to stimulation [1]. A search for the earliest responses to stimulation has focussed on the mobilization of intracellular calcium. For this purpose, the fluorescent probe chlortetracycline (CTC) has been employed in this [2–5] and other [6,7] cellular systems. CTC is reported to form fluorescent chelates with membrane-bound calcium, the mobilization of which can be monitored as a decrease in fluorescence [6,7]. In human neutrophils, the CTC fluorescence response is rapid, taking place within 5 s, making it concurrent with or faster than other known responses [1–3]. This response is also unaffected by agents which inhibit later, apparently distal, responses such as lysosomal enzyme release [2,5]. Finally, depletion of the pool of membrane-bound calcium monitored by CTC, either by prior stimulation [3] or exposure to inhibitors of glycolytic metabolism [8], results in hyporesponsiveness with respect to both the CTC response and enzyme secretion. All of these data suggest that CTC is monitoring an early, critical event in neutrophil activation.

Human neutrophils also respond to surface stimulation by a prompt, transient increment in cAMP levels [9,10]. However, the significance of this increment in stimulus–response coupling has been uncertain [9–11]. Platelets also respond to stimulation by decreases in CTC fluorescence and it has recently been reported that levels of membrane-bound calcium are modulated by cyclic nucleotides in these bodies [12]. Consequently, it was of interest to see if these agents had any effect on the CTC fluorescence response of neutrophils. In brief, we found that this response was highly sensitive to PGE_1, but not to cyclic nucleotides.

Abbreviations: CTC, chlortetracycline; fMet–Leu–Phe, *N*-formylmethionyl-leucyl-phenylalanine

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

2. MATERIALS AND METHODS

2.1. *Reagents*

Chlorotetracycline, dibutyryl-cAMP, dibutyryl-cGMP, and PGE_1 were purchased from Sigma (St Louis MO). *N*-Formyl-methionyl-leucyl-phenylalanine (fMet–Leu–Phe) was obtained from Peninsula Labs (San Carlos CA). Cytochalasin B was purchased from the Aldrich Chemical Company (Milwaukee WI).

2.2. *Procedures*

Purified preparations of human neutrophils were isolated from heparinized (10 units/ml) venous blood by means of Hypaque/Ficoll gradients [13] followed by standard techniques of dextran sedimentation and hypotonic lysis of erythrocytes [14]. The cells were washed and finally suspended in a buffer consisting of 138 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 1 mM $CaCl_2$, and 0.6 mM $MgCl_2$, pH 7.4. Lysosomal enzyme release, enzyme assays, and chlortetracycline fluorescence measurements were performed as in [2,3].

3. RESULTS

Human neutrophils were preloaded with CTC and their fluorescence response to fMet–Leu–Phe was continuously recorded. As shown in fig.1, the response to chemotactic peptide was inhibited by as little as 4 nM PGE_1; inhibition increased with prostaglandin concentration, reaching a maximum at 37–125 nM. The threshold concentration at which PGE_1 first inhibited the CTC fluorescence

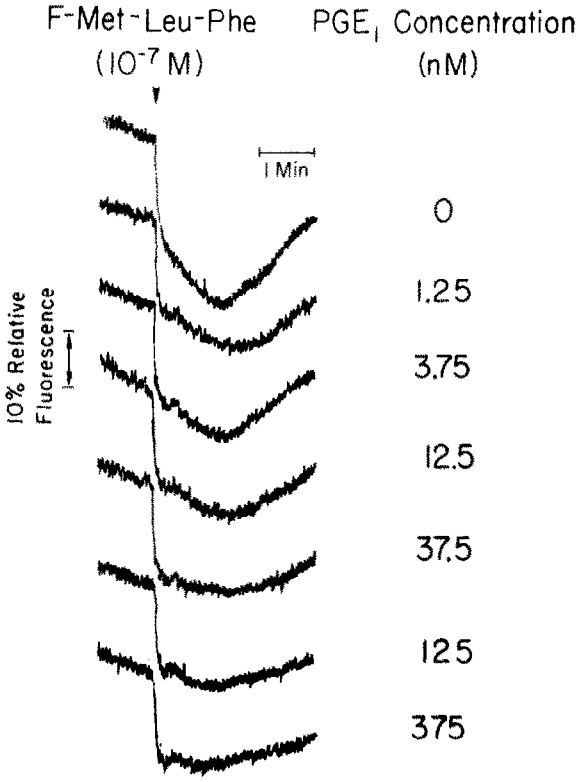

Fig.1. Effect of PGE_1 on the chlortetracycline fluorescence response to fMet–Leu–Phe. Human neutrophils, which were preloaded with CTC, were preincubated with the indicated concentrations of PGE_1 for 5 min at 37°C. At the time shown by the arrow, fMet–Leu–Phe (10^{-7} M) was added to the sample and the fluorescence was continuously recorded.

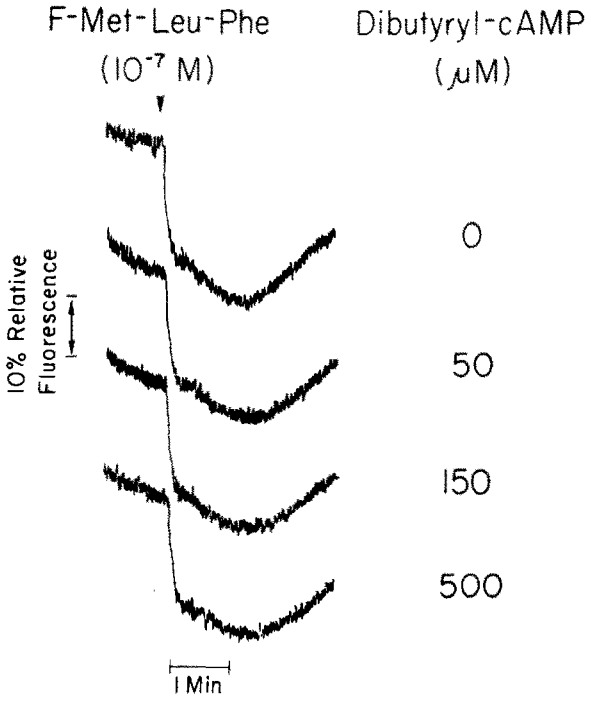

Fig.2. Effect of dibutyryl-cAMP on the chlortetracycline fluorescence response to fMet–Leu–Phe. Human neutrophils, which were preloaded with CTC, were preincubated with the indicated concentrations of dibutyryl-cAMP for 5 min at 37°C. At the time shown by the arrow, fMet–Leu–Phe (10^{-7} M) was added to the sample and the fluorescence was continuously recorded.

response was variable, with inhibition being observed at as low as 0.4 nM. High concentrations of PGE_1 produced little or no additional inhibition. Under no circumstances, even at PGE_1 concentrations as high as 250 μM, was the CTC fluorescence response abolished by this agent.

Since one of the primary means by which PGE_1 exerts its effects on cellular function is by modulating cyclic nucleotide metabolism, we examined the effect of exogenous cyclic nucleotides on the CTC fluorescence response. As shown in fig.2 and 3, both dibutyryl-cAMP and dibutyryl-cGMP were without effect upon this response. Increasing the preincubation period up to 30 min did not substantially alter these results (not shown). Failure of dibutyryl-cAMP to inhibit stimulated changes in CTC fluorescence was not affected by

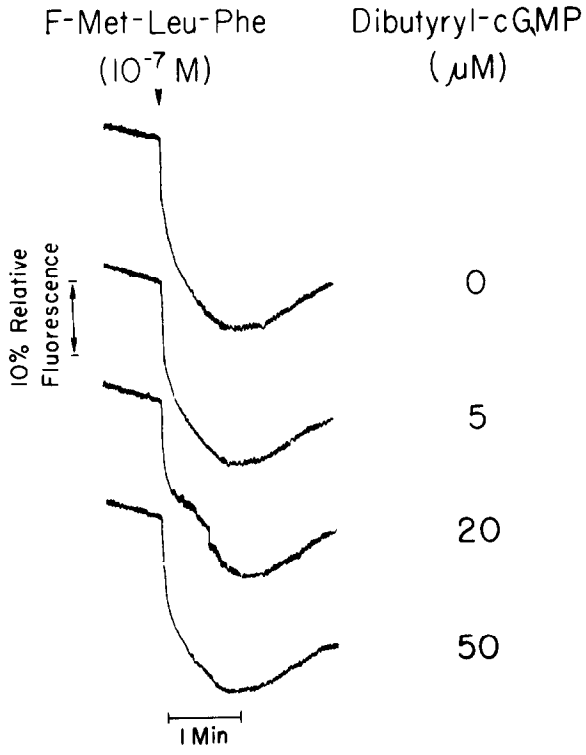

Fig.3. Effect of dibutyryl-cGMP on the chlortetracycline fluorescence response to fMet–Leu–Phe. Human neutrophils, which were preloaded with CTC, were preincubated with the indicated concentrations of dibutyryl-cGMP for 5 min at 37°C. At the time shown by the arrow, fMet–Leu–Phe (10^{-7} M) was added to the sample and the fluorescence was continuously recorded.

Table 1

Effect of cyclic nucleotides on lysosomal enzyme release

	Enzyme release (% control)	
	β-Glucuronidase	Lysozyme
No additions	(100)	(100)
Dibutyryl-cAMP		
500 μM	77.3 ± 1.6	81.7 ± 2.3
150 μM	97.7 ± 8.0	87.7 ± 13.8
50 μM	103.0 ± 7.9	94.3 ± 6.1
Dibutyryl-cGMP		
50 μM	92.8 ± 4.5	87.7 ± 6.1
15 μM	99.1 ± 16.5	95.5 ± 8.7
5 μM	97.6 ± 18.0	91.1 ± 1.5

Human neutrophils were preincubated with cytochalasin B (5 μg/ml) and the indicated concentration of cyclic nucleotide for 5 min at 37°C. The cells were stimulated with fMet–Leu–Phe (10^{-7} M) for 5 min and the results are expressed as the means (± SD; $n = 3$) of percentages of enzymes secreted by control neutrophils to which no cyclic nucleotides were added. These control cells released 39.9 ± 5.4% of their β-glucuronidase and 41.7 ± 2.0% of total lysozyme

the presence or absence of cytochalasin B and/or theophylline (not shown). That these concentrations of dibutyryl-cAMP were adequate to inhibit cellular function is shown in table 1; secretion of both β-glucuronidase and lysozyme in response to fMet–Leu–Phe were significantly inhibited by high concentrations of this agent.

4. DISCUSSION

We found that the CTC fluorescence response of human neutrophils to fMet–Leu–Phe was significantly inhibited, but not abolished, by low concentrations of PGE_1; in contrast, this response was unaffected by high concentrations of dibutyryl-cAMP. The concentrations of PGE_1 which produced a significant inhibition of this response were 2–3 orders of magnitude lower than those customarily necessary to affect neutrophil responses [11,15,16]. This dose range was also similar to that which effectively inhibited the CTC fluorescence response in platelets [12]. Thus, this effect is one of the few neutrophil responses which

can be modulated by physiologic concentrations of PGE_1 [17–19].

Other workers, using platelets, noted that inhibition of the CTC fluorescence response by PGE_1 correlated with elevated cAMP levels induced by this agent [12]. They concluded that inhibition of the fluorescence response was attributable to these elevated levels of cyclic nucleotides. Our data indicate that this mechanism is not operating in human neutrophils. The CTC fluorescence response to fMet–Leu–Phe was not affected by concentrations of dibutyryl-cAMP up to 500 M, a concentration which has been found to be effective by others [15,19–22]. From knowledge of the total cellular cAMP content [9], it can be calculated that the intracellular concentration of this nucleotide is about 1 μM. Permeation of only a small fraction of the exogenous dibutyryl-cAMP would increase the intracellular levels manifold, and exposure to this agent should be no less efficacious than pretreatment with high (micromolar) concentrations of PGE_1 [11,20]. Thus, inhibition of the CTC fluorescence response did not appear to correlate with cyclic nucleotide levels; rather, inhibition was found at very low PGE_1 concentrations which do not affect cellular responses and which do not substantially alter intracellular cAMP contents [11].

The fact that PGE_1 can inhibit the CTC fluorescence response without altering other cellular responses, such as lysosomal enzyme release, does not exclude that process monitored by the probe as an initial requirement for cell activation. The effect of increasing concentrations of PGE_1 reached a plateau at 125 nM, with higher concentrations producing no additional inhibition. Since the CTC fluorescence response was never abolished, it is likely that the remaining portion was nonetheless sufficient, either in quantity or subcellular localization, to trigger subsequent responses. Thus, these results are compatible with the hypothesis that the CTC fluorescence response measures some crucial early step in stimulus–response coupling.

In summary, we have found that stimulated CTC fluorescence responses in human neutrophils were inhibited by PGE_1 but not by dibutyryl-cAMP. These results not only dissociate the effects of this prostaglandin from those of cyclic nucleotides, but also represent one of the few known effects of PGE_1 on these cells at physiologic concentrations.

ACKNOWLEDGEMENTS

This work was supported by NIH grant AM 32471. The author is a Senior Fellow of the Arthritis Foundation.

REFERENCES

[1] Smolen, J.E., Korchak, H.M. and Weissmann, G. (1980) Inflammation 4, 145–163.
[2] Smolen, J.E. and Weissmann, G. (1982) Biochim. Biophys. Acta 208, 172–180.
[3] Smolen, J.E., Eisenstat, B.A. and Weissmann, G. (1982) Biochim. Biophys. Acta 205, 422–431.
[4] Naccache, P.H., Showell, H.J., Becker, E.L. and Sha'afi, R.I. (1978) J. Cell Biol. 83, 179–186.
[5] Naccache, P.H., Molski, T.F.P., Alobaidi, T., Becker, E.L., Showell, H.J. and Sha'afi, R.I. (1980) Biochem. Biophys. Res. Commun. 97, 62–68.
[6] Chandler, D.E. and Williams, J.A. (1978) J. Cell Biol. 76, 371–385.
[7] Chandler, D.E. and Williams, J.A. (1978) J. Cell Biol. 76, 386–399.
[8] Smolen, J.E., Noble, P., Freed, R. and Weissmann, G. (1983) submitted.
[9] Smolen, J.E., Korchak, H.M. and Weissmann, G. (1980) J. Clin. Invest. 65, 1077–1085.
[10] Simchowitz, L., Fischbein, L.C., Spilberg, I. and Atkinson, J.P. (1980) J. Immunol. 124, 1482–1491.
[11] Smolen, J.E. and Weissmann, G. (1981) Biochim. Biophys. Acta 160, 197–206.
[12] Owen, N.E. and LeBreton, G.C. (1981) Am. J. Physiol. 241, H613–H619.
[13] Boyum, A. (1968) Scand. J. Clin. Lab. Invest. 21, 77–89.
[14] Zurier, R.B., Hoffstein, S. and Weissmann, G. (1973) J. Cell Biol. 58, 27–41.
[15] Ignarro, L.J. and George, W.J. (1974) Proc. Natl. Acad. Sci. USA 71, 2027–2031.
[16] Till, G., Kownatzki, E., Seitz, M. and Gemsa, D. (1979) Clin. Immunol. Immunopathol. 12, 111–118.
[17] Wong, K. and Freund, K. (1981) Can. J. Physiol. Pharmacol. 59, 915–920.
[18] Oropeza-Rendon, R.L., Speth, V., Hiller, G., Weber, K. and Fischer, H. (1979) Exp. Cell Res. 119, 365–371.

[19] Gudewicz, P.W., Gabelman, L.B., Lai, M.Z. and Molnar, J. (1981) J. Immunopharmacol. 3, 193–204.
[20] Zurier, R.B., Weissmann, G., Hoffstein, S., Kammerman, S. and Tai, H.H. (1974) J. Clin. Invest. 53, 297–309.
[21] Kitagawa, S. and Takaku, F. (1982) Biochim. Biophys. Acta 207, 589–598.
[22] Bradshaw, D., Giroud, J.P. and Roch-Arveiller, M. (1980) Brit. J. Pharmacol. 68, 663–666.