Effect of prostaglandin E₂ and 3-morpholinosydnonimine (SIN-1) on arachidonic acid metabolism in fMLP-stimulated rat neutrophils and on thrombin-induced human platelet aggregation

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Abstract

The effects of prostaglandin (PG) E₂ and the nitric oxide (NO) donor SIN-1 on leukotriene (LT) release from formyl-methionyl-leucyl-phenylalanine (fMLP) (100 nM)-stimulated rat peritoneal neutrophils (RPN) and on thrombin-induced aggregation of washed human platelets were investigated. Both PGE₂ (1–100 nM) and SIN-1 (30–300 μM) inhibited release of LTB₄ and cysteinyl-LT from RPN in a concentration-dependent manner. The combined effects of PGE₂ and SIN-1 were not greater than expected by summation. On the other hand, the inhibitory effect of SIN-1 (0.5 or 1.0 μM) on platelet aggregation was potentiated by PGE₂ (0.3–5 μM) in a concentration-dependent manner, while PGE₂ alone in the concentrations used had only marginal effects. The results suggest differential regulation of platelet and leukocyte functions by the mediators PGE₂ and NO, which could be relevant for various physiological and pathophysiological conditions.

Introduction

Accumulation and intimate association of neutrophils and platelets have been observed in pathophysiological conditions such as the development of atherosclerotic lesions in the vessel wall [1]. The functional state of these cells may be modulated by various blood-cell- and endothelium-derived mediators [2, 3]. Both prostaglandin (PG) I₂ [4] and endothelium-derived relaxing factor (EDRF) [5], which has been identified as nitric oxide (NO) [6] or an NO-containing complex [7], have been demonstrated to inhibit platelet activation [8] and several neutrophil functions [9, 10]. In addition, synergism between PGI₂ and NO [8], iloprost and sodium nitroprusside [11], and PGE₁ and isosorbide dinitrate [12] in inhibiting platelet activation has been described. However, under normal conditions much less PGE₁ than PGE₂ is formed in human and animal tissues [13]. Similarly, lower concentrations of PGI₂ than of PGE₂ have been described to occur in inflammatory exudates [14]. Furthermore, the pharmacological effects of PGE₂ on inflammatory cells differ considerably from those of PGI₂. Thus, PGE₂ inhibits the oxygen burst and β-glucuronidase release by neutrophils stimulated with agents such as fMLP, zymosan or platelet-activating factor [15–17], while PGI₂ has much weaker effect [15, 16] or no effect at all [17]. We have now investigated the influence of PGE...
and the NO donor SIN-1 [18] on LT release from fMLP-stimulated rat neutrophils and on aggregation of thrombin-stimulated washed human platelets.

Materials and methods

Rat peritoneal exudate cells

RPN were elicited in male Wistar rats (250–300 g) by i.p. injection of 15 ml of 0.6% oyster glycogen [19]. The rats were killed 6 h later and cells were harvested by peritoneal lavage using 20 ml of ice-cold oxygenated calcium-free Tyrode solution [composition (mM): NaCl 137, KCl 2.7, NaH$_2$PO$_4$ 4.2, NaHCO$_3$ 11.9, MgCl$_2$ 1.1, glucose 5.6]. The lavage fluid was centrifuged (300 × g, 4°C, 10 min) and after hypoosmotic (0.2% NaCl) lysis of contaminating erythrocytes the RPN were washed and resuspended in ice-cold calcium-free Tyrode solution at a final concentration of 10$^7$ cells/ml. More than 90% of the cells were neutrophils and the viability was more than 98% as determined by trypan blue exclusion.

Effects of SIN-1 and PGE$_2$ on release of leukotrienes from rat peritoneal exudate cells

10$^7$ RPN were preincubated in 1 ml Tyrode solution containing 2 mM CaCl$_2$ at 37°C for 10 min in a shaking water bath. Then fMLP (final concentration 100 nM) was added and incubations were continued at 37°C for 10 min. Incubations were performed in the presence of SIN-1 (30–300 μM), PGE$_2$ (1–100 nM) or a combination of these drugs. In these experiments SIN-1 was added 5 min and PGE$_2$ 3 min before fMLP. Control incubates received appropriate volumes (50 μl) of the respective solvents. Incubations were stopped by immediate cooling in an ice-water bath and centrifugation at 1500 × g at 4°C for 3 min. Immunoreactive LTB$_4$ and cysteinyl-LT in the supernatants were determined radioimmunologically as described previously [20]. The amounts of cysteinyl-LT were calculated using standard curves for LTC$_4$ and the results were expressed in terms of immunoreactive LTC$_4$.

Platelet aggregation

Washed human platelets were prepared by the method of Radomski and Moncada [21]. Aliquots of 1 ml washed platelet suspensions (2.5 x 10$^8$ cells/ml) were incubated in a Born aggregometer under continuous stirring. After supplementation with calcium and magnesium ions (1 mM each) threshold concentrations (20–30 mU/ml) of thrombin were added and the decrease in optical density was recorded for 4 min. Some experiments were performed in the presence of SIN-1 (0.5 or 1 μM) or PGE$_2$ (0.3–5 μM) or a combination of these drugs. In these experiments PGE$_2$ was added 90 s and SIN-1 60 s before thrombin. Control incubates received the appropriate volumes (10 μl) of the respective solvents.

Statistics

Means ± SEM were calculated and statistical analysis was performed by the use of Student’s t-test for unpaired data. In the case of multigroup comparisons t-statistics were corrected by the Bonferroni method.

Materials

SIN-1 was a gift from Cassella-Riedel Pharma, Frankfurt/Main, Germany. Bovine thrombin was obtained from Behring-Werke, Marburg, Germany. fMLP, PGE$_2$, PGI$_2$ and oyster glycogen (type II) were purchased from Sigma Chemicals, St. Louis, MO, USA.
fMLP (1 mg/ml) was dissolved in dimethylsulfoxide, PGE$_2$ (10 mg/ml) in 70% ethanol, thrombin (3 U/ml) in 0.9% NaCl and SIN-1 (7.2 mM) in aqua bidest, the pH of which was adjusted to 5.2 with 0.1 N HCl. All drugs were further diluted in the incubation buffers.

Results

Drug effects on leukotriene release from rat peritoneal exudate cells

Under basal conditions, RPN released detectable amounts of LTB$_4$ (0.48 ± 0.09 ng/10$^7$ cells/10 min) into the medium, while release of cysteinyl-LT was below the detection limit of the radioimmunoassay for LTC$_4$ (<54 pg/10$^7$ cells/10 min). fMLP (100 nM) significantly stimulated the release of both LTs determined. Thus, increases in the amounts of LTB$_4$ to 12.1 ± 4.7 ng/10$^7$ cells/10 min (p < 0.001) and of cysteinyl-LT to 424 ± 129 pg/10$^7$ cells/10 min (p < 0.01) were observed.