INDUCTION OF CHEMILUMINESCENCE IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES BY THE CALCIUM IONOPHORE A23187

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1. Introduction

Human polymorphonuclear leukocytes (PMNL) exhibit a characteristic burst of oxidative metabolism following activation by suitable particles [1]. This burst is associated with the formation of several potentially bactericidal metabolites of molecular oxygen including superoxide anion, hydrogen peroxide and hydroxyl radical. Phagocytosing PMNL also generate electronically excited state(s), as manifested by the phenomenon of chemiluminescence (CL) [2]. It was proposed that relaxation of singlet oxygen (to its triplet ground state) and/or relaxation of excited carbonyl moieties formed during the respiratory burst may provide a basis for this phenomenon. The formation of singlet oxygen via the myeloperoxidase (MPO)–hydrogen peroxide–halide reaction has been demonstrated [3], which is operative in phagocytosing PMNL. Moreover, analysis of the CL spectra for the MPO–H_2O_2–halide and PMNL reactions are reportedly quite similar [4].

In our laboratory we have been examining the relationship between CL and oxidative metabolism in human PMNL through the use of several soluble agents known to induce a respiratory burst in these cells in the absence of particles. One such agent is the divalent cation ionophore A23187, which has been reported to stimulate oxidative metabolism in resting neutrophils in a calcium-dependent manner [5,6]. This communication describes the calcium-dependent induction of CL in resting human PMNL by ionophore A23187 and the ability of this agent to modify phagocytosis-associated CL induced by opsonized zymosan.

2. Materials and methods

2.1. *Ionophore*

The carboxylic acid ionophore A23187 was the generous gift of Dr R. L. Hamill of Eli Lilly and Co., Indianapolis. A 4 μM solution of this agent was prepared immediately prior to use by dissolving 0.1308 mg in 20 μl dimethyl sulfoxide and then diluting appropriately in Dulbecco's phosphate buffer solution (PBS, Grand Island), pH 7.4, containing 0.1% glucose. The final concentration of DMSO was $< 0.04\%$ and was without effect.

2.2. *Leukocyte isolation*

PMNL were isolated by dextran sedimentation [7] from peripheral venous blood obtained from healthy adult volunteers. Lysis of residual erythrocytes was accomplished by brief (2 min) exposure to 0.83% ammonium chloride. The leukocyte pellet was rinsed once in PBS and then resuspended in 1–2 ml buffer. PMNL yields were determined by hemacytometer and the suspensions adjusted to 2×10^7/ml.

2.3. *Particle opsonization*

Zymosan A (Sigma Chemical Co., St Louis) 20 mg, was suspended in a few ml of 0.9% NaCl and placed in a boiling water bath for 30 min. Following centrifugation at $500 \times g$ for 5 min, the zymosan pellet was resuspended in 5 ml plasma (previously rendered free of cellular debris by centrifugation) and incubated at 37°C for 30 min. The opsonized zymosan was washed once in PBS and resuspended to 4 mg/ml.

2.4. *Leukocyte viability*

Cellular integrity of PMNL exposed to ionophore A23187 (1 μM) was assessed by monitoring release of the cytoplasmic enzyme lactate dehydrogenase, according to [8].

2.5. *Chemiluminescence*

CL responses were monitored using a Packard ambient temperature liquid scintillation counter (Model 2002) operated in the out-of-coincidence mode. Gain was set at 100% and the discriminator at 0-1000. Leukocytes (10^7 cells) and PBS were added to dark-adapted plastic liquid scintillation vials and incubated in a 37°C water bath for 15 min under constant agitation. The vials were individually removed from the water bath, blotted gently and background CL monitored for 0.5 min intervals. The vials were then immediately returned to the water bath until the next counting interval. CL responses were initiated via the addition of either 2 mg opsonized zymosan, A23187 (1 μM final) or both. CL was then monitored at 1, 5, 10, 15 and 20 min intervals. All vials contained a total reaction volume of 2 ml.

2.6. *Statistical analysis*

Comparisons of peak CL responses were performed by paired analysis using a two-tailed *t*-test.

3. Results

Figure 1 depicts the CL responses of human PMNL exposed to the calcium ionophore A23187 and/or opsonized zymosan. Addition of the ionophore alone to resting PMNL induced a CL response in these cells (16 071 ± 4275 net counts/0.5 min, mean ± SE for 5 exp.) at a concentration (1 μM) which was non-cytotoxic. The ionophore-induced CL response was considerably less than that observed following addition of opsonized zymosan to PMNL. Nevertheless, addition of A23187 and zymosan simultaneously to resting PMNL resulted in an augmented response to zymosan.

Table 1 shows the effect of deleting calcium ions from the external milieu on the peak CL responses generated by PMNL exposed to A23187, opsonized zymosan, or A23187 plus zymosan. The ability of the ionophore to induce CL in resting PMNL was

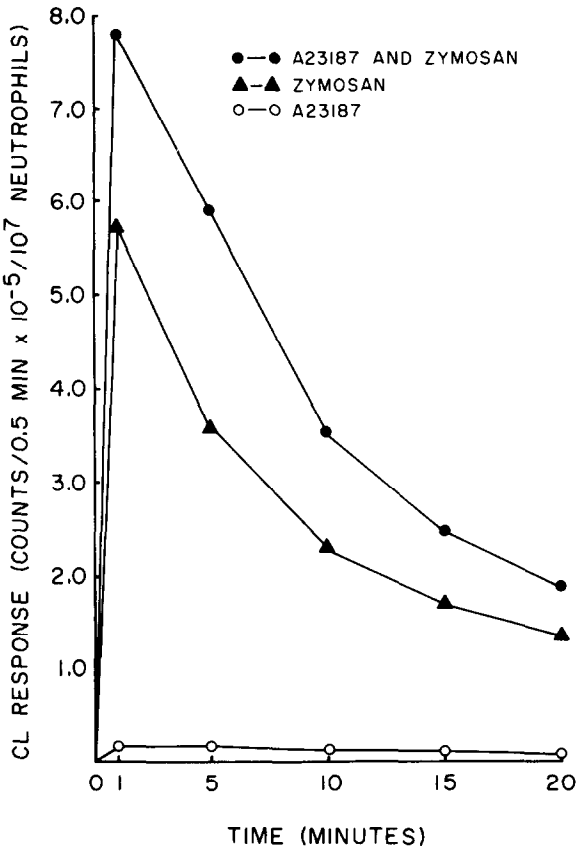

Fig.1. Chemiluminescence responses of human PMNL exposed to A23187, opsonized zymosan, or A23187 plus zymosan. Each point represents the mean value of single determinations from 5 separate experiments. The final concentrations of A23187 and opsonized zymosan are 1 μM and 1 mg/ml, respectively.

significantly impaired when extracellular calcium was absent. In contrast, phagocytosis-associated CL induced by zymosan alone or zymosan plus A23187 did not require extracellular calcium. The inability of PMNL to respond to the ionophore alone in calcium-free buffer containing magnesium ions suggests that magnesium is unable to substitute for calcium ions in this reaction. In fact, CL responses induced by A23187 are greater in buffer containing calcium alone than in buffer containing calcium and magnesium ions (data not shown). This suggests that magnesium ions may compete with calcium ions for complexation by the ionophore.

Table 1
Influence of extracellular calcium on A23187- and zymosan-induced CL responses

	Peak CL response (counts/0.5 min)[a,b]		Statistical comparison
	with calcium (0.9 mM $CaCl_2$)	without calcium (0.49 mM $MgCl_2$)	
Ionophore A23187	16 071 ± 4275	1906 ± 420	$p < .005$
Opsonized zymosan	571 933 ± 46 458	577 018 ± 102 531	NS
A23187 plus zymosan (simultaneously added)	782 005 ± 124 169	684 277 ± 93 026	NS

[a] Mean ± standard error, $n = 5$
[b] All values are corrected for background CL

4. Discussion

This resport describes the calcium-dependent induction of chemiluminescence in resting human polymorphonuclear leukocytes exposed to the divalent cation ionophore A23187. In addition to inducing a CL in resting PMNL, the ionophore was also observed to augment phagocytosis-associated CL evoked by opsonized zymosan. Earlier studies have demonstrated the ability of this agent to stimulate oxidative metabolism in PMNL independently of phagocytosis in a calcium-dependent manner [5,6,9]. Another soluble agent, phorbol myristate acetate (PMA), was reported to stimulate oxidative metabolism and CL in resting PMNL [10]. The ability of soluble agents such as PMA and A23187 to induce respiratory activity and CL generation in PMNL independently of phagocytosis lends support to the contention that membrane perturbation may constitute the primary stimulus for metabolic activation of these cells.

The requirement for extracellular calcium in the ionophore-induced CL responses of resting PMNL led us to examine the role of extracellular calcium in the CL responses of PMNL to other activating agents. It was observed that phagocytosis-associated CL evoked by zymosan, in contrast to A23187-induced CL, does not require the presence of extracellular calcium. In further studies (unpublished observations) we have also found that PMA-induced CL responses are independent of a requirement for extracellular calcium. It is likely, therefore, that if calcium ions are involved in mediating and/or modulating the oxidative metabolic activity of PMNL, mobilization of intracellular calcium pool(s) rather than influx of extracellular calcium may be required. The ability of the ionophore to stimulate a respiratory burst in these cells could arise from the displacement of intracellular (presumably bound) calcium by calcium being transported into the cell by the ionophore. The existence of an intracellular calcium pool(s) has not been conclusively demonstrated in PMNL to date. However, it has recently been reported that PMNL prelabeled with ^{45}Ca releases this cation when activated by opsonized zymosan and that this efflux paralleled release of a granule-associated enzyme, β-glucuronidase [11]. Clearly, further studies are required to elucidate both role of calcium ions in the metabolic responses of PMNL and the subcellular location of intracellular calcium pool(s).

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