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Stimulation of intracellular Ca^{2+} elevation in neutrophils by thiol-oxidizing phenylarsine oxide

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Abstract

Phenylarsine oxide (PAO), a trivalent arsenical compound, stimulated $[Ca^{2+}]_i$ elevation in rat neutrophils in a Ca^{2+}-containing medium but caused no appreciable response in a Ca^{2+}-free medium. PAO also induced external Mn^{2+} entry, which was inhibited by *N*-acetyl-L-cysteine (NAC), but failed to elicit any appreciable Ba^{2+} and Sr^{2+} entry. Pretreatment of neutrophils with thiol-reducing agents including dithiothreitol (DTT), NAC, 2,3-dimercapto-1-propanol (DMP), 2,3-dimercaptopropane-1-sulfonic acid (DMPS) and tris-(2-carboxyethyl)phosphine (TCEP), all greatly inhibited PAO-induced $[Ca^{2+}]_i$ elevation. Addition of Ni^{2+} or La^{3+} followed by PAO stimulation also attenuated the Ca^{2+} signals in a concentration-dependent manner. PAO had no significant effect on the production of reactive oxygen intermediates (ROI) and nitric oxide (NO) nor did it decrease cellular low molecular weight thiols levels. PAO-induced $[Ca^{2+}]_i$ elevation was significantly inhibited by 1-[6-[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (U-73122), the inhibitor of phospholipase C-coupled processes, genistein, a general tyrosine kinase inhibitor, phorbol 12-myristate 13-acetate (PMA), a protein kinase C (PKC) activator, calyculin A, a cortical actin stabilizer, 2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one (LY 294002), a phosphoinositide 3-kinase inhibitor, 1-[β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1*H*-imidazole (SKF-96365), and *cis*-*N*-(2-phenylcyclopentyl)azacyclotridec-1-en-2-amine (MDL-12,330A), the blockers of receptor-gated and store-operated Ca^{2+} channels, whereas there was no appreciable effect exerted by aristolochic acid, a phospholipase A_2 inhibitor, 7-nitroindazole and *N*-(3-aminomethyl)benzylacetamidine (1400W), the blockers of NO synthase, and by suspension in a Na^+-deprived medium. In contrast, 2-aminoethoxydiphenyl borane (2-APB), the blocker of IP_3 receptor and Ca^{2+} influx, enhanced the PAO-induced response. PAO had no effect on the plasma membrane Ca^{2+}-ATPase (PMCA) activity in the pharmacological isolated neutrophil preparation and the neutrophil membrane fractions. These results indicate that PAO stimulates $[Ca^{2+}]_i$ rise in rat neutrophils mainly through the oxidation of vicinal thiol groups on the cell surface membrane to activation of a non-store operated Ca^{2+} entry (non-SOCE) without affecting the activity of PMCA and the plasmalemmal Na^+/Ca^{2+} exchanger.

Keywords: Phenylarsine oxide; Cation entry; Intracellular free-Ca^{2+}; Signal transduction; Non-store operated Ca^{2+} entry; Neutrophils

Abbreviations: 1400W, *N*-(3-aminomethyl)benzylacetamidine; AA, arachidonic acid; 2-APB, 2-aminoethoxydiphenyl borane; CPA, cyclopiazonic acid; DAF-2/DA, 4,5-diaminofluorescein diacetate; DMP, 2,3-dimercapto-1-propanol; DMPS, 2,3-dimercaptopropane-1-sulfonic acid; DTT, dithiothreitol; GEA3162, 5-amino-3-(3,4-dichlorophenyl)1,2,3,4-oxatriazolium; HBSS, Hanks' balanced salt solution; H_2DCF/DA, 2′,7′-dichlorodihydrofluorescein diacetate; IP_3, D-*myo*-inositol 1,4,5-trisphosphate; LY 294002, 2-(4-morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one; mBBr, monobromobimane; MDL-12,330A, *cis*-*N*-(2-phenylcyclopentyl)azacyclotridec-1-en-2-amine; NAC, *N*-acetyl-L-cysteine; NO, nitric oxide; PAO, phenylarsine oxide; PIK3, phosphoinositde-3-kinase; PKC, protein kinase C; PLC, phospholipase C; PMA, phorbol 12-myristate 13-acetate; PMCA, plasma membrane Ca^{2+}-ATPase; ROI, reactive oxygen intermediates; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SKF-96365, 1-[β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1*H*-imidazole; SOCE, store-operated Ca^{2+} entry; TCEP, tris-(2-carboxyethyl)phosphine; U-73122, 1-[6-[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione

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1. Introduction

Ca^{2+} signals have been implicated in many cellular functions of neutrophils. The increase in $[Ca^{2+}]_i$ involves two mechanisms: Ca^{2+} release from internal stores and Ca^{2+} entry across the plasma membrane. It is well established that the receptor-mediated initial Ca^{2+} spike is mediated by the activation of phosphoinositide-specific phospholipase C (PLC) that hydrolyzes membrane phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) to generate the second messenger, D-*myo*-inositol 1,4,5-trisphosphate (IP_3), which interacts with IP_3 receptor on the internal stores for the release of Ca^{2+} [1]. However, the mechanism regulating Ca^{2+} influx across the plasma membrane, which accounts for the sustained increase in $[Ca^{2+}]_i$, is still unclear. In non-excitable cells, including neutrophils, depletion of the intracellular Ca^{2+} stores induces entry of Ca^{2+} across the plasma membrane, referred to as SOCE (capacitative Ca^{2+} entry) [2]. Several hypotheses have been considered for the mechanism of SOCE. Recently, a secretion-like coupling model based on a physical and reversible trafficking of portions of the endoplasmic reticulum toward the plasma membrane has been proposed [3], which is supported by the dynamic cytoskeletal structure. It is far from certain that this mechanism is the only one involved in the increase in Ca^{2+} entry in non-excitable cells. A non-store operated Ca^{2+} entry (non-SOCE) mechanism that involves protein kinase C (PKC) has been reported in human platelets [4]. The $PI(3,4,5)P_3$-sensitive Ca^{2+} entry that is independent of the filling state of internal Ca^{2+} stores was observed in FcεRI-stimulated mast cells [5]. In addition, arachidonic acid (AA) activates the non-SOCE in smooth muscle cells [6]. The thiol modification of a number of important membrane proteins or channels induced Ca^{2+} entry in neutrophils through a non-SOCE mechanism [7,8].

Phenylarsine oxide (PAO) is a membrane-permeable trivalent arsenical compound, covalently binding the vicinal thiol groups of proteins that are in suitable proximity to form stable ring structures [9], and has been reported to inhibit phosphotyrosine phospha tases [10], the superoxide anion generation [11], and to induce L-selectin shedding [12] in neutrophils. PAO increased in $[Ca^{2+}]_i$ in a number of cell types including endothelial cells [13], platelets [14], T cells [15], and macrophages [16], and inhibited Ca^{2+} entry via N-type Ca^{2+} channel in neuromuscular junctions [17]. Our recent reports indicated that *N*-ethylmaleimide and 5-amino-3-(3,4-dichlorophenyl)1,2,3,4-oxatriazolium (GEA3162) induced Ca^{2+} entry in neutrophils through thiol-modification [7,8]. The aim of this study was to examine the effect of PAO on Ca^{2+} signaling in rat neutrophils.

2. Materials and methods

2.1. Materials

Dextran T-500 was purchased from Amersham Pharmacia Biotech. Hanks' balanced salt solution was obtained from Invitrogen. Fluo-3/AM, fura-2/AM, 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF/DA), tris-(2-carboxyethyl)-phosphine (TCEP) and monobromobimane (mBBr) were purchased from Molecular Probes. 2-(4-Morpholinyl)-8-phenyl-4*H*-1-benzopyran-4-one (LY 294002) was obtained from Biomol Research Laboratories. Cyclopiazonic acid (CPA), 1-[6-[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione (U-73122), 4,5-diaminofluorescein diacetate (DAF-2/DA), 2-aminoethoxydiphenyl borane (2-APB), 7-nitroindazole, 1-[β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1*H*-imidazole (SKF-96365), 1400W, calyculin A and oligomycin A were obtained from Calbiochem-Novabiochem. *cis*-*N*-(2-Phenylcyclopentyl)azacyclotridec-1-en-2-amine (MDL-12,330A) and GEA3162 were purchased from Alexis. All other reagents and chemicals were purchased from Sigma–Aldrich. The final volume of DMSO in the reaction mixture was $\leq$0.5% (v/v).

2.2. Preparation of rat neutrophils

Blood was collected from the abdominal aorta of male Sprague–Dawley rats (3 months old) and the neutrophils were purified by dextran sedimentation, centrifugation through Ficoll–Paque, and hypotonic lysis of erythrocytes [7]. Purified neutrophils containing >95% viable cells were normally resuspended in Hanks' balanced salt solution (HBSS) containing 10 mM HEPES, pH 7.4, and 4 mM $NaHCO_3$, and kept in an ice-bath before use. All experiments in the present study were performed under the guidelines of the Institutional Experimental Laboratory Animal Committee and were in strict accordance with the principles and guidelines contained in the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health.

2.3. Measurement of intracellular free Ca^{2+}

Neutrophils (5×10^7 cells/ml) were incubated with 5 μM fluo-3/AM for 45 min at 37 °C. After being washed, the cells were resuspended in HBSS to 5×10^6 cells/ml. In some experiments, cells were suspended in Na^+-deprived HEPES buffer (124 mM *N*-methyl-D-glucamine, 4 mM KCl, 0.64 mM K_2HPO_4, 0.66 mM KH_2PO_4, 10 mM HEPES, pH 7.4, 5.56 mM dextrose, and 15.2 mM $KHCO_3$). Fluorescence changes were monitored with a