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EXPERIMENTAL PAPER

Synthetic inotropes inhibit the expression of adhesion molecules and augment the expression of L-selectin in polymorphonuclear neutrophils☆

B. Trabold*, M. Gruber, D. Fröhlich

Klinik für Anästhesiologie, Universität Regensburg, 93042 Regensburg, Germany

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Summary
Objectives: To elucidate differential functional and phenotypic changes in response to clinically relevant synthetic inotropes plus the generation of oxidative free radicals by polymorphonuclear neutrophils (PMN), and changes in the expression of L-selectin and Mac-1 on the surface of PMN were examined in the presence of dobutamine and dopexamine in pharmacological concentrations.
Design: Prospective, in vitro study.
Setting: Research laboratory.
Subjects: Human PMN obtained from healthy donors.
Interventions: PMN were pretreated with dobutamine 147.99 nM or 147,990 nM, or dopexamine 100 nM or 100,000 nM, followed by stimulation with FMLP. Stimulated neutrophils were incubated with antibiodies against CD11b or CD62l and assessed by flow cytometry. Additional probes were assessed by flow cytometry for the generation of oxidative free radicals.
Measurements and main results: Low concentrations of both synthetic inotropes significantly inhibit the suppression of CD62l expression following stimulation with *N*-formyl-*l*-methionyl-*l*-leucyl-*l*-phenylalanine; high concentrations antagonize this effect. High concentrations of both synthetic inotropes suppresses the expression of CD11b. Neither dobutamine nor dopexamine modified the generation of oxidative free radicals.
Conclusions: While the upregulation of Mac-1 expression is inhibited in a dose-dependent manner, the expression of L-selectin is enhanced at low concentrations of dobutamine and dopexamine and partly counter-regulated at high concentrations. It seems that synthetic inotropes can modulate the immunomodulatory ability by inhibition of PMN rolling and modification of PMN adherence and diapedese.

☆ A Spanish translated version of the summary of this article appears as Appendix in the final online version at 10.1016/j.resuscitation.2007.01.010.

* Corresponding author. Tel.: +49 941 9447801; fax: +49 941 9447802.
E-mail address: benedikt.trabold@klinik.uni-regensburg.de (B. Trabold).

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Introduction

The use of synthetic inotropes remains a mainstay in the treatment of acutely ill patients suffering from life-threatening conditions like cardiac failure or septic shock. Even if in septic shock, as opposed to cardiac shock, the uptake of oxygen and the utilisation in mitochondria is impaired, the goal of therapy with synthetic inotropes remains to increase the oxygen delivery to physiological or so-called ''supranormal'' values. Despite the haemodynamic effect of inotropes, endocrine catecholamines, like adrenaline (epinephrine) or dopamine, affect the behaviour of different immunomodulatory cells, including polymorphonuclear neutrophils (PMN).[1–3]

Regardless of its ability to generate oxidative burst responses, PMN transmigration is a pivotal step in countering infectious agents. The multi-step process of transmigration involves cell rolling, adhesion on endothel and transmigration of PMN. The PMN rolling is mediated by expression of L-selectin (CD62l) on the surface of PMN. Adherence and diapedesis of PMN is mediated by β_2 integrins which consist of two subunits. The β-subunit consist of three parts: CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), and CD11c/CD18 (p150).

Despite the known effects of adrenaline or dopamine on PMN, to our knowledge no investigation has compared the effects of clinically relevant synthetic inotropes on the functional and morphological changes in PMN. The aim of the present investigation was to test the hypothesis that exposure of synthetic inotropes to stimulated PMN modulates the expression of L-selectin and Mac-1 on the surface of PMN. Furthermore, to elucidate whether synthetic inotropes modified the ability of PMN to destroy pathogens, we studied the capacity of PMN to generate reactive oxygen specimens. For this reason, we investigated the reactive oxygen production of PMN and the changes in expression of L-selectin and Mac-1 on the surface of PMN following exposure to dobutamine and dopexamine. Functional and morphological changes of PMN can be induced by a variety of agents such as *N*-formyl-*l*-methionyl-*l*-leucyl-*l*-phenylalanine (FMLP) and phorbol 12-myristate 13-acetate (PMA). FMLP is a bacterial peptide from *Escherichia coli*. FMLP exerts its effects through a specific G-protein-coupled receptor of the neutrophil. In contrast, PMA stimulates PMNs by a direct activation of protein kinase C bypassing post-receptor signalling. PMA can therefore serve as a positive control of maximum stimulation.

Materials and methods

Neutrophils

After obtaining informed consent whole blood was drawn from 10 healthy donors with no history of infection 2 weeks before the experiments. The study was approved by the local ethics board of the University of Regensburg.

PMN isolation and cell preparation

Heparinised (10 U ml^{-1}) whole blood (3 ml) was layered on top of 3 ml Histopaque® (Sigma, Deisenhofen, Germany). Erythrocytes aggregated at the interface and settled at room temperature. After 30 min leukocyte-rich plasma was withdrawn with care to avoid contamination of plasma with Histopaque®. To avoid artefactual activation of cells, the isolation process did not involve lysis, centrifugation, or washing procedures.

H_2O_2 production

The leukocyte-rich plasma was mixed in PBS (4 μl leukocytes + 100 μl Dulbecco's PBS) and incubated for 20 min at 37 °C. The solution was loaded with the fluorogenic substrates dihydrorhodamine 123 (DHR; 2 μl) and carboxy-seminaphthorhodafluor-1-acetoxymethylester (SNARF/AM; 2 μl) (both Molecular Probes, Eugene, OR, USA). Following further incubation for 10 min at 37 °C, one of the following substances was suspended: dobutamine (Hexal, Holzkirchen, Germany) 147.99 nM or 147,990 nM, or dopexamine (Zeneus, Oxford, UK) 100 nM or 100,000 nM, or terbutaline (Stada, Bad Vilbel, Germany) 20 nM or 20,000 nM, or PBS for control. After incubation for 10 min at 37 °C, 100 nM FMLP (Sigma Chemicals, Deisenhofen, Germany) or 100 nM PMA was added. Finally, after further incubation (15 min, 37 °C), dead cells were counterstained with 30 μM propridium iodide (Serva, Heidelberg, Germany). The samples were then placed on ice to stop any further reaction.

Immunostaining

The leukocyte-rich plasma was mixed 1:50 in PBS (20 μl leukocytes + 980 μl Dulbecco's PBS). After incubation for 20 min at 37 °C one of the following substances was suspended: dobutamine 147.99 nM or 147,990 nM, or dopexamine 100 nM or 100,000 nM, or terbutalin 20 nM or 20,000 nM, or PBS. Following further incubation for 20 min at 37 °C 100 nM FMLP or 100 PMA was added to stimulate the PMN. The solution was then