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High-Dose Catecholamine Treatment Decreases Polymorphonuclear Leukocyte Phagocytic Capacity and Reactive Oxygen Production

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Flow cytometry was used to study phagocytic function (uptake of fluorescein isothiocyanate-labeled bacteria) and release of reactive oxygen products (dihydrorhodamine 123 converted to rhodamine 123) following phagocytosis by neutrophil granulocytes of heparinized whole blood treated with adrenaline, noradrenaline, dopamine, dobutamine, or orciprenaline. Reduced neutrophil phagocytosis and reactive oxygen production were seen at 12 μg of adrenaline per liter (72% each compared with control values); at 120 μg of noradrenaline (72% each), dobutamine (83 and 80%, respectively), and orciprenaline (81 and 80%, respectively) per liter; and at 100 μg of dopamine per liter (66 and 70%) ($P < 0.05$ for all). At these dosages, neutrophil chemotaxis was reduced to <50% of control values for all catecholamines. Treatment with catecholamines at lower dosages had no significant effect on phagocytosis or generation of reactive oxygen products or chemotaxis. The phagocytic capacity of granulocytes was related to the generation of reactive oxygen products ($r = 0.789$; $P < 0.05$). The results demonstrate that catecholamines have a suppressive effect on the response of phagocytic cells to bacterial pathogens at high therapeutic levels in blood.

Polymorphonuclear leukocytes (PMNLs) and their mediators have long been recognized to play a crucial role in generating, maintaining, or even worsening the clinical aspects of infectious diseases (3, 7, 19). An impairment of PMNL function results in an impaired host defense against invading microorganisms (13, 18), facilitating the onset of infections.

In the intensive care unit setting, the clinical term "systemic inflammatory response syndrome" summarizes life-threatening diseases such as polytrauma and hemorrhage, septicemia, and severe pancreatitis. Frequently, catecholamines are administered to patients with these conditions to maintain adequate organ perfusion, and various infections are well-recognized clinical problems in intensive care units.

Catecholamines have been demonstrated to inhibit chemotactic responses of PMNLs (6, 9, 22). To elucidate whether catecholamines (adrenaline, noradrenaline, dopamine, dobutamine, and orciprenaline) also influence PMNL phagocytic ability and/or reactive oxygen production, we examined the phagocytic function and release of reactive oxygen products in heparinized whole blood treated with these catecholamines using a flow cytometry assay and compared the results with those of chemotaxis assays (9, 20, 21).

MATERIALS AND METHODS

Materials. Adrenaline (Hoechst, Frankfurt, Germany), noradrenaline (Boehringer, Ingelheim, Germany), dopamine (Nattermann, Cologne, Germany), dobutamine (Lilly, Vienna, Austria), and orciprenaline (Boehringer) were added to heparinized whole-blood samples from 12 healthy subjects (age, 24 to 32 years) at concentrations ranging between 1.2 and 120 mg/liter, and the mixtures were incubated for 1 h at room temperature. Neutrophil counts were in the range of 4.6×10^{12} to 6.1×10^{12}/liter (59 to 69%) with 0.2×10^{12} to 0.4×10^{12} (3 to 4%) band forms per liter. No catecholamines were added to the control samples.

Heat-killed *Escherichia coli* ATCC 25922 cells (10^8/ml) were labeled with fluorescein isothiocyanate as previously described (20, 21). Dihydrorhodamine 123 was purchased from Molecular Probes, Inc. (Eugene, Oreg.) and dissolved in *N*,*N*-dimethyl formamide (Sigma Chemicals, Munich, Germany) at a concentration of 3 μg/ml.

Phagocytosis assay. To 100 μl of heparinized whole blood (as controls, test tubes with different catecholamines were used), 10 μl of precooled fluorescein isothiocyanate-labeled *E. coli* was added, and the mixture was incubated for 10 min at 37°C. Thereafter, 100 μl of precooled quenching solution (Orpegen, Heidelberg, Germany) was added, and the samples were washed twice in phosphate-buffered saline (PBS) (pH 7.4). Finally, 2 ml of fluorescence-activated cell sorter (FACS) lysing solution (Becton Dickinson, Vienna, Austria) was added. After 20 min, the samples were washed again, resuspended in 100 μl of PBS containing propidium iodide (PI) at a concentration of 50 μg/ml for DNA staining, and kept on ice until analysis.

Reactive oxygen intermediate (ROI) production. Blood samples were stimulated with 25 μl of *E. coli* (10^8/ml) (ATCC 25922, not labeled) at 37°C. After 10 min, 25 μl of the dihydrorhodamine 123 solution was added. After another 10 min at 37°C, 2 ml of FACS lysing solution was added and the mixture was incubated for 20 min at room temperature. Thereafter, the samples were washed with PBS and resuspended in 100 μl of PBS containing PI at a final concentration of 50 μg/ml for DNA staining.

Flow cytometry. The cells were analyzed on a standard FACScan flow cytometer (Becton Dickinson). For each measurement, 10,000 events were collected. To exclude cell debris and nonphagocytized bacteria, a gate was set on PI-stained leukocytes during acquisition in FL2 (red). For analysis of ROI production, the shift to the right in FL1 (green) was determined. The amount of cleaved substrate was estimated from the mean fluorescence by using the statistical option of the FACScan software.

Similarly, the amount of phagocytized bacteria was assessed by a shift in mean fluorescence to the right (FL1). The proportion of phagocytic cells in the granulocyte population was shown to be more than 90% (21). The mean fluorescences of the two assays were compared with that of unstimulated controls.

To evaluate whether subpopulations of PMNLs are more responsive to catecholamine treatment, cell populations were determined by using phycoerythrin-conjugated CD14, CD18, and CD11a (Biomedica, Vienna, Austria) and CD11c and CD56 (Becton Dickinson) monoclonal antibodies. A phycoerythrin-conjugated anti-immunoglobulin G1 (anti-IgG1) antibody (Biomedica) was the control. Each catecholamine was administered at a concentration of 120 ng/ml. Briefly, 20 μl of each monoclonal antibody was added to the test tubes, which were then kept for 15 min at 4°C in the dark after phagocytosis quenching or

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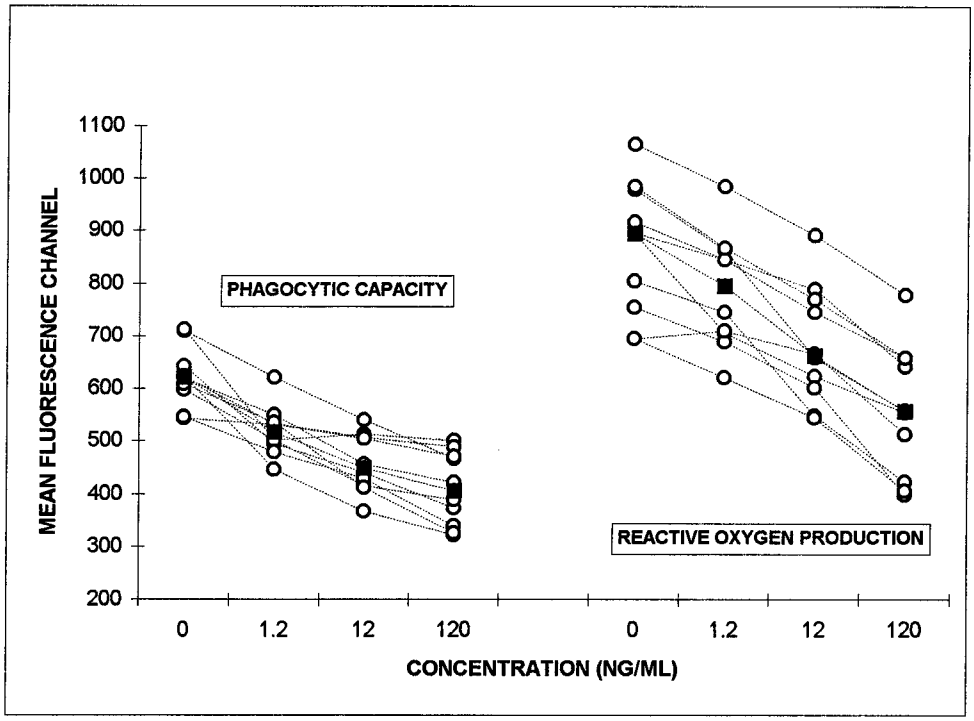

FIG. 1. Effect of adrenaline on PMNL phagocytic ability and reactive oxygen production. ■, median.

dihydrorhodamine 123 oxidation. Thereafter, samples were treated in the absence of PI as described above (FACS lysing solution and PBS washing, etc.).

Chemotaxis assay. PMNLs were isolated as described elsewhere (5). Briefly, 4 ml of venous whole blood was hemolyzed with 16 ml of isotonic ammonium chloride at 4°C for 15 min. Thereafter, the cells were washed three times and resuspended in PBS. The chemotaxis assay was a modification of the procedure of Boyden (9). A 10-μl sample of the PMNLs and lipopolysaccharide (LPS) (10 μl, 200 μg/ml) or PBS (negative control) was placed on a chemotaxis plate containing 45 ml of double-distilled water, 8 ml of medium 199 plus HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) (Sigma, Irvine, United Kingdom), 3 ml of 10% fetal calf serum (Scandic, Vienna, Austria), and 15 ml of agarose (Behring, Marburg, Germany). The catecholamines remained in contact with the PMNLs during the 90-min incubation at 37°C with 5% CO_2. After fixation with 10% methanol and 10% formaldehyde (Sigma), the PMNLs were stained with Giemsa solution (Merck, Darmstadt, Germany). The migration distances were measured with a digital cell viewer (Biegler, Mauerbach, Austria) and expressed in millimeters. Differences in comparison with the PMNLs without catecholamine treatment were calculated as percentages.

Statistical methods. For comparisons of test and control samples, Kruskall-Wallis and Mann-Whitney-Wilcoxon U tests were used. Different concentrations of antimicrobial agents were compared by using the Wilcoxon rank sum test. For correlation analysis, Spearman's test was used. All the analyses were two-sided, and differences with a P of < 0.05 were considered statistically significant.

RESULTS

Effects of catecholamines on PMNL phagocytic ability. The tested catecholamines decreased PMNL phagocytic ability in a dose-dependent manner. Reduced neutrophil phagocytosis was seen at 12 μg of adrenaline per liter (72% compared with the controls); at 120 μg of noradrenaline (72%), dobutamine (83%), and orciprenaline (81%) per liter; and at 100 μg of dopamine per liter (66%) ($P < 0.05$ for all) (Fig. 1 to 5).

Effects of catecholamines on PMNL reactive oxygen production. Similarly to PMNL phagocytic ability, ROI production decreased in a catecholamine dose-dependent manner. Reduced neutrophil reactive oxygen production was seen at 12 μg of adrenaline (72% compared with the control value) and noradrenaline (72%) per liter, at 120 μg of dobutamine (80%) and orciprenaline (80%) per liter, and at 100 μg of dopamine per liter (70%) ($P < 0.05$ for all). Prior to catecholamine treatment, PMNL phagocytic ability correlated with ROI production ($r = 0.764$; $n = 14$). However, after catecholamine treatment, even at a low dose (1.2 ng/ml), no relation between PMNL phagocytosis and ROI production was found.

Effects of catecholamine treatment on PMNL subpopulations. Tables 1 and 2 depict the effects of catecholamine treatment on PMNL phagocytosis and reactive oxygen production, respectively, in distinct subpopulations (CD56, CD14, CD18, CD11a, and CD11c) expressed as percent differences from the IgG1 control values. The maximal difference in phagocytic ability between the subpopulations and the IgG1 controls was 19% to −15% in the samples without catecholamine incubation (Table 1). Similarly, the maximal difference in PMNL reactive oxygen production between the subpopulations and the IgG1 controls was −14% in the untreated group (Table 2). Thus, smaller differences were regarded as nonsignificant.

Effects of catecholamine treatment on PMNL chemotaxis. Incubation with adrenaline, noradrenaline, dopamine, dobutamine, and orciprenaline resulted in an inhibition of the chemotactic response. The migration distances were reduced to 37, 30, 23, 24, and 46% of the control distances, respectively.

DISCUSSION

Gram-negative bacteria and their cell wall constituents (LPS, endotoxin) and cytokines can elicit the release of catecholamines in vivo (10, 15, 17). Catecholamines have a bimodal effect on LPS-induced tumor necrosis factor (TNF) production, the eventual result depending on whether the α- or β-adrenergic receptor is preferentially stimulated (12, 14).

Knowledge of the effect of catecholamines on PMNL function in sepsis is important not only because feedback loops

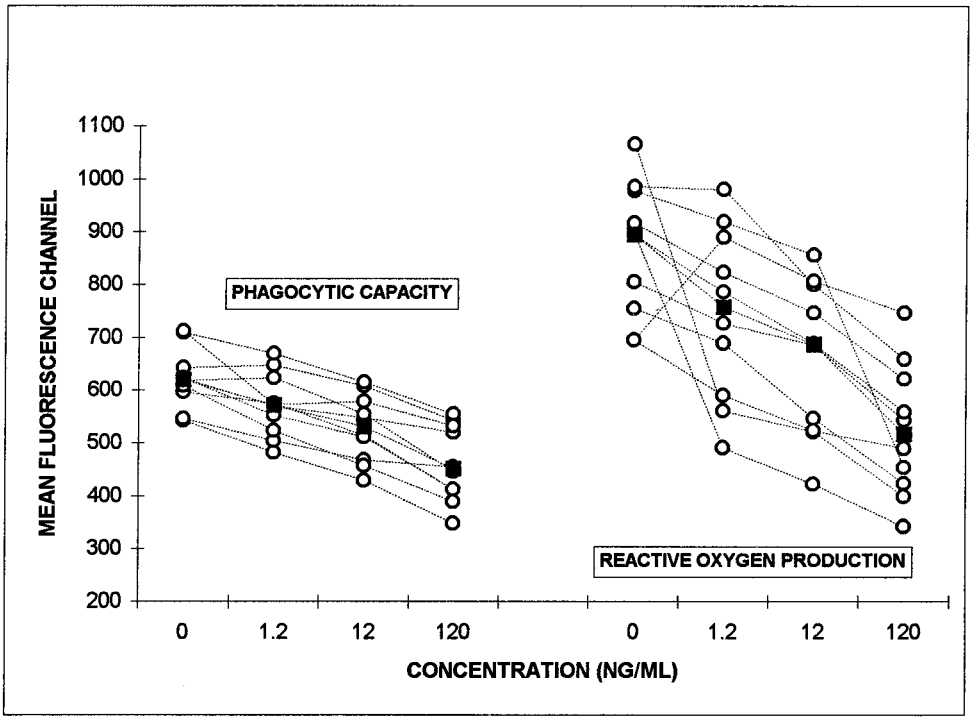

FIG. 2. Effect of noradrenaline on PMNL phagocytic ability and reactive oxygen production. ■, median.

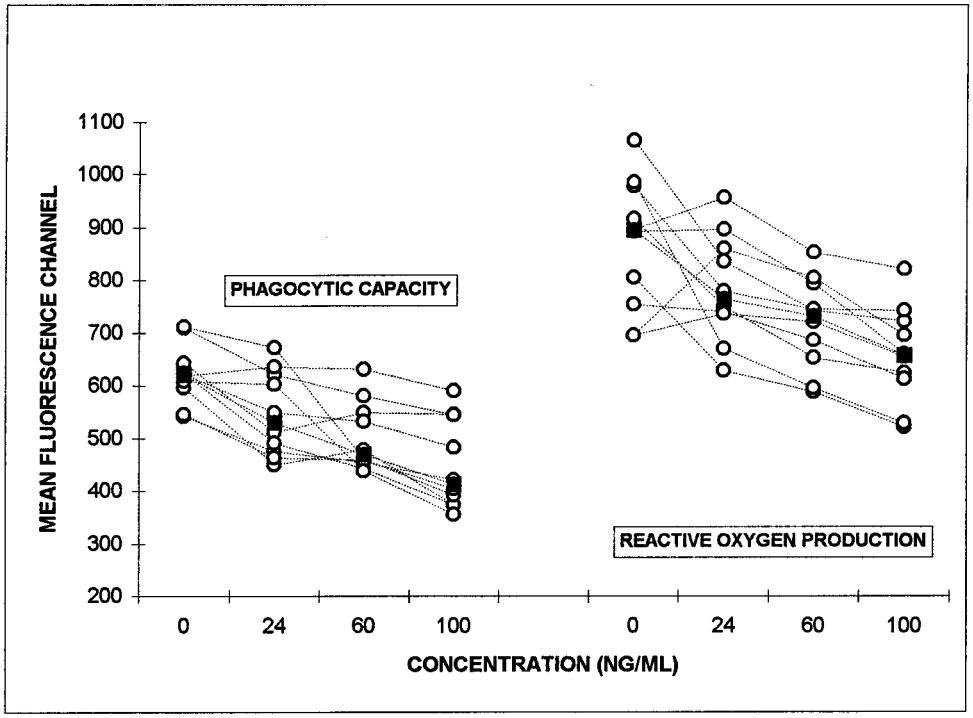

FIG. 3. Effect of dopamine on PMNL phagocytic ability and reactive oxygen production. ■, median.

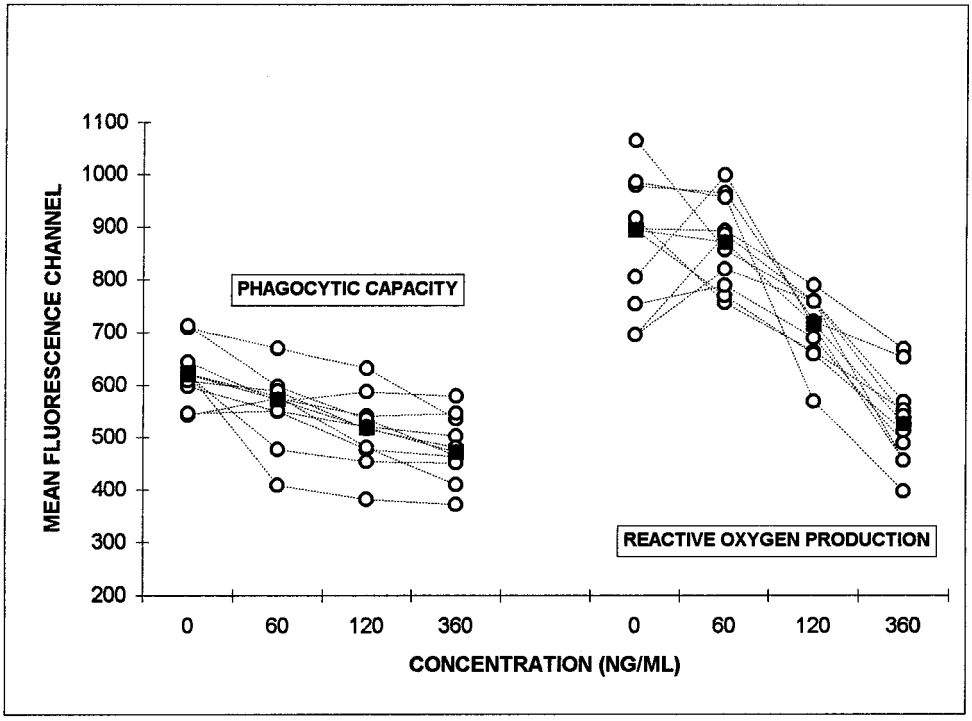

FIG. 4. Effect of dobutamine on PMNL phagocytic ability and reactive oxygen production. ■, median.

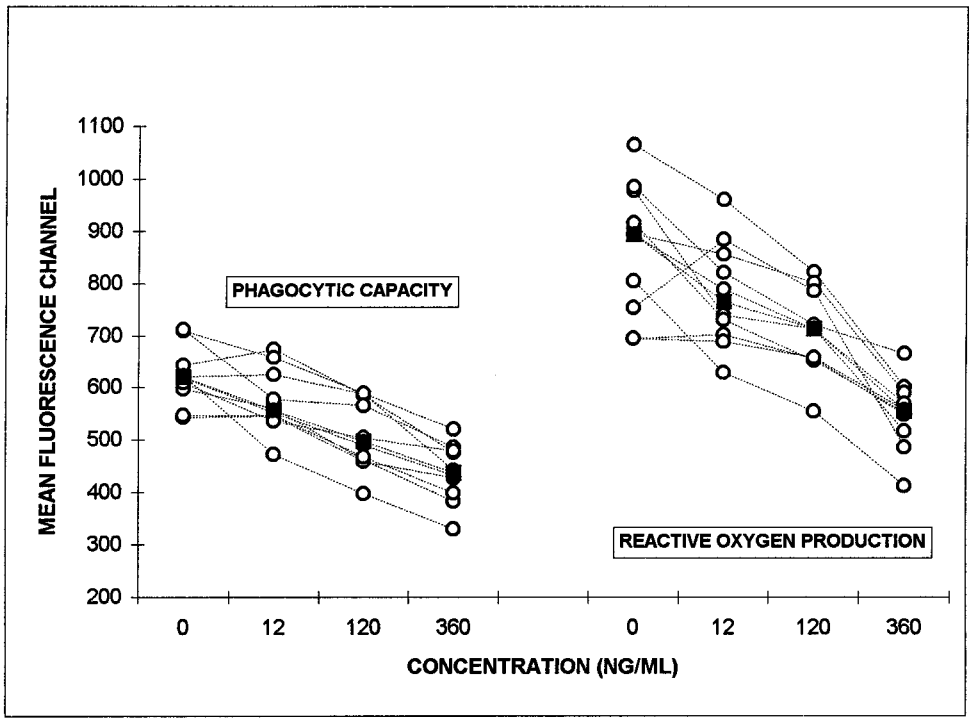

FIG. 5. Effect of orciprenaline on PMNL phagocytic ability and reactive oxygen production. ■, median.

TABLE 1. Effects of catecholamine treatment on PMNL phagocytosis in distinct subpopulations

Catecholamine	Effect on phagocytosis in the indicated subpopulation[a]				
	CD56	CD14	CD18	CD11a	CD11c
None	−15	11	19	4	−6
Adrenaline	−15	−15	−5	16	15
Noradrenaline	7	−14	−15	0	10
Dopamine	−4	−9	−2	8	12
Dobutamine	4	−12	−9	−3	8
Orciprenaline	0	−16	−10	12	−4

[a] Expressed as percent differences from the IgG1 control values.

between these cells and their environment are poorly understood but also because catecholamines are frequently administered to patients with septic shock as vasoactive substances to maintain organ perfusion.

This study aimed to assess the effects of various clinically administered catecholamines (adrenaline, noradrenaline, dopamine, dobutamine, and orciprenaline) on PMNL function after *E. coli* challenge in human whole blood. To evaluate whether subpopulations of PMNLs are more responsive to catecholamine treatment, cell populations were determined by using anti-CD14, -CD18, -CD11a, -CD11c, and -CD56 monoclonal antibodies.

An ex vivo system that has been shown to be a suitable model for study of PMNL function in sepsis has been applied (20, 21). The use of whole blood eliminates artifacts that may be associated with the isolation of PMNLs, such as adherence-induced upregulation of TNF expression (8). Additionally, by using whole blood, endocrine effects on PMNL functional responses after *E. coli* challenge can be investigated under conditions of a physiological background, which is likely to be of more relevance for the in vivo situation.

The present results demonstrate that the applied catecholamines depress PMNL phagocytic ability and intracellular ROI production. In the experiments with the monoclonal antibodies, no catecholamine-hyperresponsive or -hyporesponsive PMNL subpopulation was detected. In addition, PMNL chemotaxis was reduced to 20 to 40% of that of the controls. In granulocytes, most functions are inhibited by cyclic AMP (2). There is evidence that TNF priming of neutrophils for enhanced agonist (neutrophil-activating peptide 2 and interleukin 8 [IL-8]) activity involves the antagonistic down-modulation of stimulus-induced rises in cyclic AMP (1). Catecholamines were shown to increase cyclic AMP (9). Altogether, our findings suggest a generalized, nonspecific inhibitory effect of catecholamines on PMNL function.

Recently, catecholamines have been demonstrated to suppress TNF and IL-6 release in a similar whole-blood test system (16). This effect was thought to inhibit the ongoing production of these mediators in sepsis as part of a negative feedback loop.

Catecholamines could reduce PMNL function via indirect and direct mechanisms. Since TNF and IL-6 are known to prime neutrophils for enhanced phagocytosis and respiratory burst (21), the feedback mechanism could indirectly lead to a depressed function in situations in which excessive amounts of catecholamines are given to maintain cardiovascular function.

In addition, a direct modulation of PMNL function via α- and β-adrenergic receptors is possible. This seems to be more unlikely, since factors which are extrinsic and not intrinsic to the neutrophil are the crucial determinants of their function (4, 11, 21).

In conclusion, the results demonstrate that catecholamines per se depress PMNL phagocytic ability and ROI production at high but clinically achievable dosages. In situations of depressed PMNL function, such as septicemia (11, 13), a further depression via catecholamines could contribute to unfavorable outcome (mortality of ≥40%). Like cytokines, catecholamines are plasma factors capable of influencing PMNL function in septicemia (21).

TABLE 2. Effects of catecholamine treatment on PMNL reactive oxygen production in distinct subpopulations

Catecholamine	Effect on reactive oxygen production in the indicated subpopulation[a]				
	CD56	CD14	CD18	CD11a	CD11c
None	−3	−1	−3	−14	−4
Adrenaline	0	−1	0	0	0
Noradrenaline	1	0	0	1	1
Dopamine	0	2	0	0	0
Dobutamine	0	1	0	0	1
Orciprenaline	4	−2	0	−1	0

[a] Expressed as percent differences from the IgG1 control values.

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