Pathophysiological Aspects of Functional Modulation of Human Peripheral Blood Neutrophils with Propranolol

V. A. Frolov, E. G. Moiseeva, and A. V. Pasechnik

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We studied the effect of β-adrenoceptor antagonist propranolol on the regulation of spontaneous apoptosis in neutrophils, priming of lipopolysaccharide-treated neutrophils, and expression of neutrophil adhesion factors. The influence of propranolol on apoptosis, adhesion, and generation of oxygen radicals by neutrophils was shown to be an additional mechanism of the action of β-adrenoceptor antagonists. This pathophysiological mechanism probably mediates the effect of neuroendocrine transmitters and explains the role of adrenergic antagonists in the pathogenesis and therapy of inflammation, cardiovascular diseases, and bronchial asthma.

Key Words: *neutrophils; propranolol; adhesion; apoptosis; reactive oxygen species*

Pathophysiological symptoms of inflammation in vascularized tissues are associated with molecular, biochemical, and cellular response to microbial invasion. These complex, interrelated, and sometimes excessive processes are stereotypic. Much attention is paid to selective inhibition of adrenergic activation as a new form of modulation of the inflammatory process. The effects of adrenergic antagonists are now extensively studied. Neutrophils play an important role in the human organism under normal and pathological conditions, which is related to multiple functions of these cells [7]. Neutrophils are short-lived cells. They circulate in the vascular bed for 10 h. After apoptosis, these cells are phagocytized by monocytes or migrate into tissues. Cell death occurs after 4-5 days [2]. Specific cell receptors play a key role in the regulation of neutrophil function. These receptors are involved in adhesion, apoptosis, generation of reactive oxygen species (ROS), and activation of neutrophils under the influence of lipopolysaccharide (LPS) [2]. Neutrophils express membrane β-adrenoceptors, which contributes to regulation of neutrophil function by the neuroendocrine system (in addition to the immune system) [3]. Little is known about the role of β-adrenoceptors in activation of innate immunity and regulation of apoptosis in human neutrophils.

Here we studied the effect of β-adrenoceptor antagonist propranolol on the regulation of spontaneous apoptosis in neutrophils, priming of LPS-treated neutrophils, and expression of neutrophil adhesion factors.

MATERIALS AND METHODS

Peripheral blood neutrophils of healthy donors were isolated on a Ficoll-Verografin gradient [1]. The cells were washed 2 times with phosphate buffered saline (pH 7.4). To study apoptosis, the cells (1×10^6 cells/ml) were cultured in RPMI 1640 medium with 10% fetal bovine serum (Sigma), 1% L-glutamine, and 1% penicillin and streptomycin (culture medium) at 37°C and 5% CO_2 for 9 h. The ratio of apoptotic and living cells was estimated by the method of flow cytometry using Hoechst-33258 fluorescent probe [4]. Viability of cultured cells was 95-98%.

Neutrophil adhesion was studied by counting nonadherent neutrophils and expressed in percents. The maximum number of adherent cells was taken

Russian People's Friendship University

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as 100%. The reaction was conducted in 96-well flat-bottom plates. Neutrophils (0.2×10^6 cells/ml) and propranolol in various concentrations were diluted with physiological saline and put in a well. The cells were cultured in the medium at 37°C and 5% CO_2 for 60 min.

ROS generation by neutrophils was studied by the chemiluminescence method [1]. Neutrophils (0.2×10^6 cells/ml) were placed in Hanks medium containing Ca^{2+}, Mg^{2+}, and luminol (3.5×10^{-4} M). Eppendorf tubes were maintained in a 1250 LKB luminometer cell under temperature-controlled conditions. ROS generation by neutrophils was activated by phorbol-12-myristate-13-acetate (PMA, 100 nM).

The data are presented as *M±SD* (independent experiments). The significance of differences was evaluated by Student's *t* test.

RESULTS

Adhesion increased insignificantly under the influence of propranolol in concentrations of 0-100 μM. Propranolol in concentrations of 100-400 μM increased adhesion to 100% (Fig. 1). ROS generation by neutrophils was activated by phorbol ester (Fig. 2). Propranolol in a concentration of 200 μM suppressed ROS generation in control cells (Fig. 2, *2*) and primed neutrophils (20-min incubation with LPS) treated with PMA (Fig. 2). In the presence of propranolol ROS generation by primed neutrophils (Fig. 2, *4*) was similar to that observed in experiments with cells not exposed to priming and propranolol treatment (Fig. 2). Incubation of neutrophils with propranolol in concentrations of 0-400 μM dose-dependently increased the rate of spontaneous apoptosis. The saturation concentration of propranolol was 200 μM. In the presence of propranolol the number of cells with degraded DNA was 59% (*vs.* 39% in the control, Fig. 3).

The interaction of propranolol with neutrophils is accompanied by inhibition of protein kinase C and phosphatidic acid phosphohydrolase [5]. LPS increases adhesion and contributes to the respiratory burst in neutrophils. p38 mitogen-activated protein kinase (p38MAPK) plays an important role in these processes [4]. The increase in neutrophil adhesion involves CD18 β2-integrins, whose activation accelerates apoptosis in cells [6]. These data suggest that the increase in the ratio of apoptotic cells under the influence of propranolol is related to activation of β2-integrins.

Our results indicate that propranolol increases adhesion and stimulates apoptosis in cultured neutrophils, which is probably associated with activation of β2-integrins.

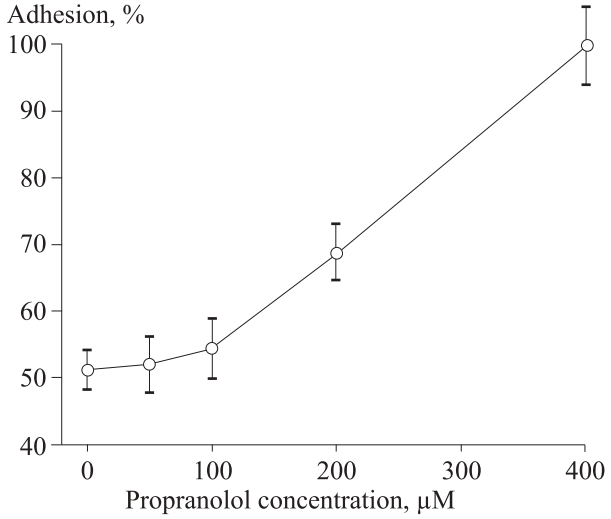

Fig. 1. Effect of propranolol on adhesion of human neutrophils.

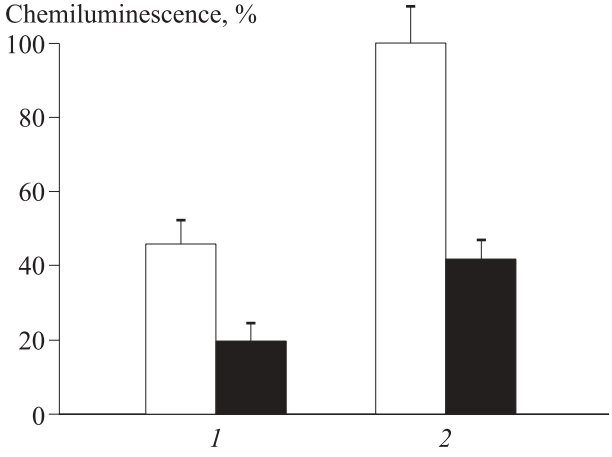

Fig. 2. Effect of propranolol on ROS generation by neutrophils in the absence (*1*) or presence of LPS (*2*). Samples: 2×10^5 neutrophils, 350 μM luminol, and Hanks medium. Light bars, control; dark bars, propranolol.

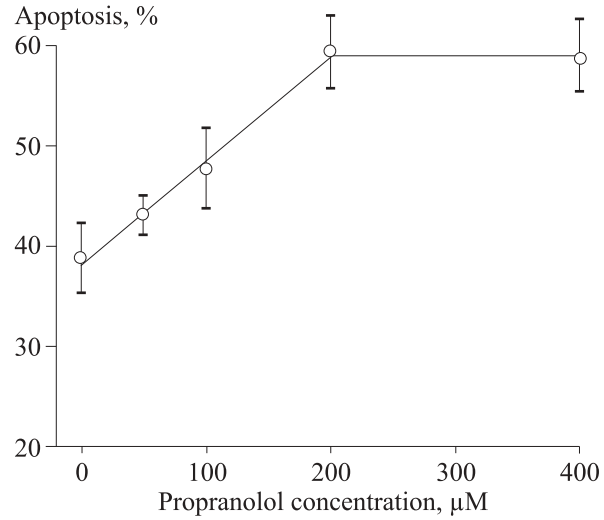

Fig. 3. Effect of propranolol on apoptosis of human neutrophils.

Functional dichotomy of helper activity in T lymphocytes is modified by catecholamines and adrenergic antagonists. These changes are manifested in modulation of 2 restricted clones producing interleukins and regulating the pathological process. This specific feature of cytokines provides the optimum immune response of the cytokine network. It involves neutrophils, monocytes, and cascade regulatory interleukins (IL-4 and IL-10) dependent on T helper cells of types 1 and 2 [9].

Our results indicate that adrenoceptors on phagocytic cells serve as a target for the early influence of the activated sympathetic system and therapeutic action of adrenergic antagonists. These drugs alter sensitivity of the adrenergic system and dependent functions. Activation of adrenoceptors is associated with the self-regulatory process of receptor desensitization.

REFERENCES

1. M. G. Vinokurov, N. I. Kosyakova, N. N. Nikolaeva, *et al*., *Biofizika*, **44**, 929-930 (1999).
2. N. A. Mayanskii, M. I. Zaslavskaya, and A. N. Mayanskii, *Immunologiya*, No. 2, 11-13 (2000).
3. M. Bergmann and T. Sautner, *Wien Klin. Wochenschr*., **114**, 752-761 (2002).
4. P. A. Detmers, D. Zhou, E. Polizzi, *et al*., *J. Immunol*., **161**, No. 4, 1921-1929 (1998).
5. S. Dunzendorfer and C. J. Wiedermann, *J. Mol. Cell. Cardiol*., **32**, 915-924 (2000).
6. J. Larsson, L. Serrander, O. Stendahl, *et al*., *Inflam. Res*., **49**, 452-459 (2000).
7. L. A. O'Reilly and A. Strasser, *Ibid*., **48**, 5-21 (1999).
8. M. A. Park, M. J. Lee, S. H. Lee, *et al*., *FEBS Letters*, **519**, 45-49 (2002).
9. M. Wahle, T. Greulich, C. G. Baerwald, *et al*., *Immunobiology*, **210**, No. 1, 43-52 (2005).