Effect of Activators on Neutrophil Mobility

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Effect of various activators on chaotic movement of neutrophils on the glass is studied using a Magiscan 2A automatic image analysis system. All tested agents in concentration inducing neutrophil activation suppress their mobility, but no one completely inhibits neutrophil motion. The cells retain the ability to change their shape and partially to move.

Key Words: *neutrophil mobility; neutrophil activation; image analysis*

Neutrophils are polyfunctional mobile cells responding to activating stimuli by secretion of granules. There are few published data on the relationship between neutrophil movement and their activation.

The aim of the present study was to evaluate the effect of activators of different chemical structures on chaotic neutrophil motion. We studied the effect of activators acting via specific plasma membrane receptors, such as concanavalin A (ConA), *Phaseolus vulgaris* phytohemagglutinin (PHA), lipopolysaccharide from *E. coli* (LPS), and activators acting via nonreceptor pathways (calcium ionophore A23187 and phorbol ester, protein kinase C activator). All activators were tested in concentrations, which according to published data induce neutrophil activation.

Additionally, the effect of dibutyryl analog of cyclic adenosine monophosphate (cAMP), which induces no neutrophil stimulation, was studied.

MATERIALS AND METHODS

Neutrophils were isolated from blood samples obtained from fingers of healthy donors. Several blood drops were transferred onto a small cover glass and placed into a humidified thermostat for 10-15 min. The glass was washed with Hanks' solution to remove erythrocytes and clots and transferred to a slide. A contour of mineral oil corresponding to the size equal to the cover glass was preliminary made on the slide and filled with Hanks' solution containing 1% human serum albumin. The mount was additionally covered with a large cover glass, so that the cells lie within a closely sealed chamber.

Conditioned medium was the standard Hanks' solution supplemented with 1% human serum albumin (Fluka), pH 7.3 at 37°C. All tested activators in a required concentration were added to this medium.

Neutrophil mobility on the glass was assessed using a Magiscan 2A image analysis system in dark field of a Jenalumar microscope with objectives ×6.3 and ×16 [1,2,4].

Cell images were isolated using a standard Magiscan 2A image analysis software, cell movements were monitored using a shot-by-shot cell identification software developed by us. The record for evaluating the rate of neutrophil movement consisted of 20 subsequent pictures at 1-min intervals followed by mathematical processing of the records. Motion-related changes in cell area were assessed at shot-by-shot intervals of 2 sec [1]. Simultaneous tracking of many cells in the field of view allowed us to evaluate the mobility of both individual cells and the whole cell population.

Neutrophil behavior was assessed by the mean rate of cell movement in a population of 40-70 cells and motion-related changes in individual neutrophil area.

The effects of activators were assessed in flow chambers where the medium could be completely replaced for 1 min.

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Most experiments were carried out in sealed chambers, but at least 2 experiments with each activator were performed in flow chambers.

The following activators were tested: 10^{-6} M formyl-peptide (formyl-Met-Leu-Phe, Calbiochem), 100 μg/ml ConA, 100 μg/ml PHA, 60 μg/ml LPS, 10^{-6} M calcium ionophore A23187, 10^{-5} M phorbol ester, and 5×10^{-5} M dibutyryl cAMP. Other agents were from Sigma.

RESULTS

It has been previously shown that the main rate of neutrophil movement in a population is a constant parameter that characterizes population mobility [2]. The baseline neutrophil motion rate measured in 40 healthy donors was 8.8±0.2 μ/min (from 7 to 11 μ/min).

Effect of receptor activators. Figure 1, *a* presents the time course of the mean distances passed by all cells in the population in the absence and presence of 10^{-6} M formyl-peptide. Formyl-peptide sharply reduced the slope of this curve, which indicates pronounced and sustained inhibition of neutrophil movement.

Formyl-peptide reduced the rate of neutrophil movement by on average 4 μ/min, and this effect persisted for about 1 h.

In flow-chambers we observed a sharp and sustained inhibition of neutrophil motion after addition of ConA and PHA. On the contrary, LPS induced a slow and progressive inhibition of neutrophil motion for 40-90 min (Fig. 1, *b*). In 2 out of 5 experiments with LPS we first observed a rise of neutrophil motion rate followed by its inhibition.

Data presented in Table 1 suggest that the lectins ConA and PHA markedly suppressed cell mobility, while LPS produced the slightest effect.

Effect of secondary transmitters. Elevation of intracellular concentration of cyclic nucleotides in experiments is usually achieved by using their butyryl derivatives. These substances are more lipophilic than nonacylated nucleotides and more readily penetrate through the plasma membrane and are rapidly deacylated in the cells [5].

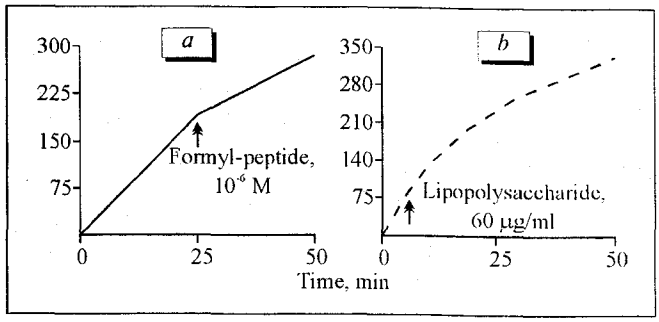

Fig. 1. Time course of the mean distances traveled by all cells in the population in the absence and presence of formyl-peptide (*a*) and lipopolysaccharide (*b*). Ordinate: trace, μ. Abrupt sustained (*a*) and smooth (*b*) suppression of cell mobility.

Experiments in flow chambers showed that the calcium ionophore A23187 smoothly inhibited cell movement. The phorbol ester-induced inhibition was abrupt in 3 and smooth in 2 experiments. Dibutyryl cAMP had an abrupt effect on neutrophil mobility. The rate of neutrophil motion was measured 0.5-1 h after addition of test substances (Table 2).

As seen from Table 2, A23187, phorbol ester, dibutyryl cAMP exert similar effects on cell mobility. None of these agents immobilized neutrophils completely; moreover, the cells actively changed their shape. These changes are seen in cell area plots (Fig. 2). Figure 2 illustrates the effect of ConA, which in our experiments was the most potent inhibitor of cell motion.

The mechanism by which activation processes in neutrophils are associated with suppression of undirected cell motion remain unknown. The most popular concept is that activators, in particular formyl-

TABLE 1. Rate of Neutrophil Movement in the Presence of Formyl-Peptide, ConA, PHA, and LPS

No. of experiment	Mean rate of neutrophil motion, μ/min			
	formyl-peptide, 10^{-6} M	ConA, 100 μg/ml	PHA, 100 μg/ml	LPS, 60 μg/ml*
1	3.5	3.3	3.9	4.8
2	6.0	2.7	3.6	6.3
3	3.6	1.9	4.1	2.5
4	5.6	2.5	3.5	6.6
5	4.1	3.1	3.4	5.9
6	4.5			
Mean (*M*±*m*)	4.5±0.425	2.7±0.245	3.7±0.130	5.2±0.745

Note. *40-50 min after addition.

TABLE 2. Rate of Neutrophil Movement in the Presence of Secondary Transmitters (μ/min)

No. of experiment	A23187, 10^{-6} M	Phorbol ester, 10^{-5} M	cAMP, 5×10^{-5} M
1	6.0	5.5	5.4
2	3.5	5.1	5.5
3	5.0	2.1	6.0
4	4.3	4.0	3.5
5	4.9	3.5	4.9
6	5.3	4.2	4.7
Mean ($M\pm m$)	4.8±0.350	4.1±0.494	5.0±0.354

peptide and phorbol ester, improve cell adhesion to the substrate [11].

Neutrophil stimulation by different activators is always associated with generation of superoxide anion and cell degranulation. These processes are closely coupled and only few examples of their uncoupling have been reported [6,13]. We proceeded from the assumption that neutrophil is a polyfunctional cell characterized by mobility and secretion. These functions cannot be executed simultaneously without impairing one of them.

Changes in neutrophil mobility induced by different concentrations of formyl-peptide are a typical biphasic reaction. A threshold concentration inducing chemotaxis and chemokinesis is about 10^{-9} M, while a peak concentration inducing maximum chemotaxis and chemokinesis is 10^{-8} M, which is also a threshold concentration for neutrophil degranulation. Increasing the concentration of formyl-peptide leads to suppression of neutrophil motion and enhances their degranulation [10].

It is now commonly accepted that mobility of nonmuscular cells is structurally ensured by cell cytoskeleton consisting of actin, myosin, tubulin, and a great variety of actin-binding proteins [12]. The role of cytoskeleton in calcium-dependent secretion of cell granules is now well established [3]. Recent published data indicate that in neutrophils cell mo-

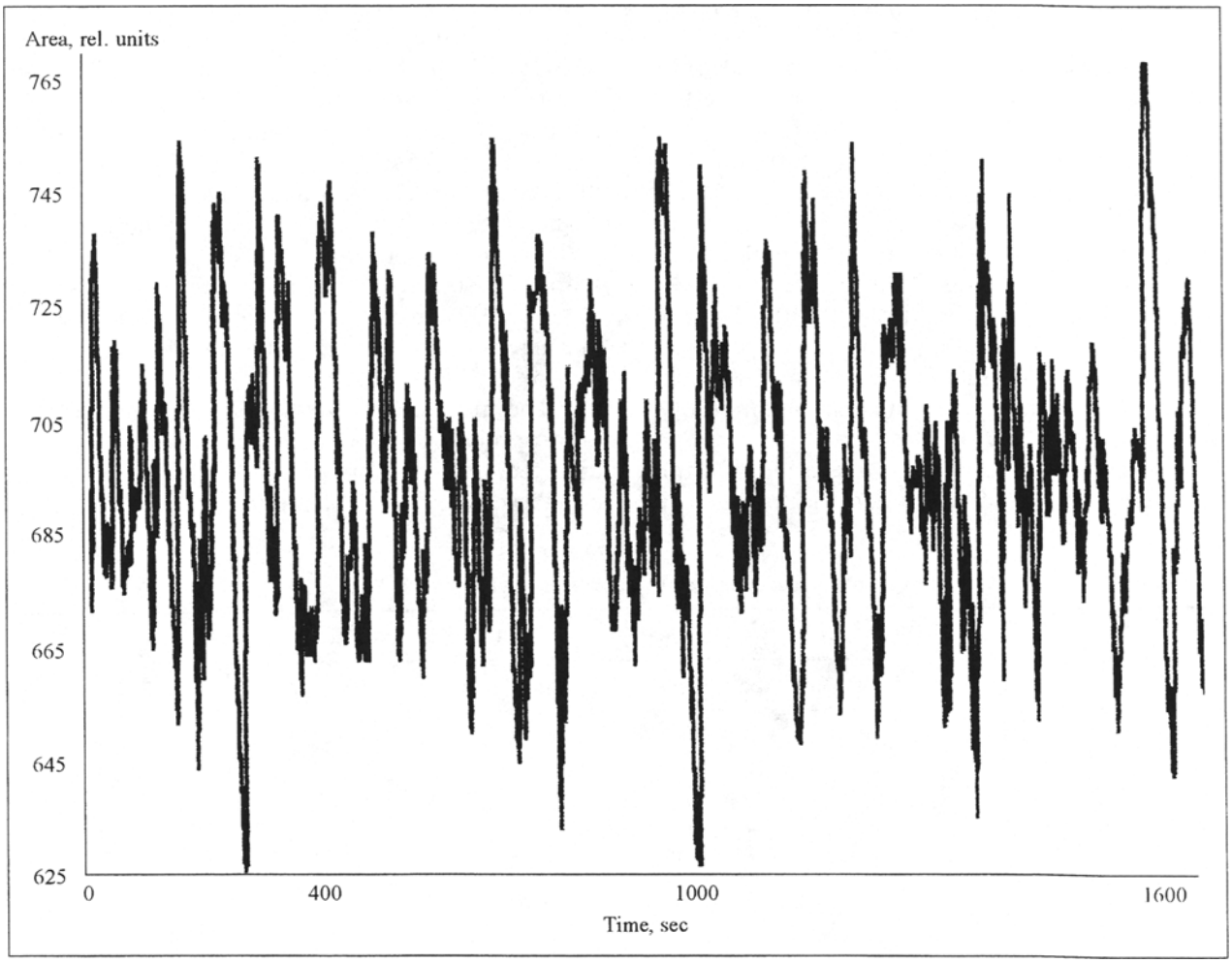

Fig. 2. Changes in neutrophil area in the presence of concanavalin A (100 μg/ml). Despite marked suppression of cell mobility, the cell actively changes its shape.

bility and exocytosis are calcium-independent processes [7,8]. The cytoskeleton is involved in translocation of secretory granules and neutrophil degranulation.

We assume that suppression of neutrophil mobility during activation is inhibited because the cytoskeleton is switched from motion to secretion.

Unlike well-known neutrophil activators stimulating secretion, cAMP not only suppresses neutrophil mobility but also blocks its activation [9]. The regulatory effect of cAMP is based on inhibition of common neutrophil structures which are responsible for both neutrophil motion and secretion.

REFERENCES

1. A. A. Galkin, E. A. Tumanov, E. N. Timin, *et al.*, *Vopr. Med. Khimii*, No. 6, 7-10 (1994).
2. A. A. Galkin, E. A. Tumanov, M. M. Filippov, *et al.*, *Byull. Eksp. Biol. Med.*, **111**, No. 5, 491-495 (1991).
3. R. H. Glebov, *Endocytosis and Exocytosis* [in Russian], Moscow (1987).
4. E. A. Tumanov, A. A. Galkin, M. M. Filippov, *et al.*, *Byull. Eksp. Biol. Med.*, **109**, No. 6, 594-597 (1990).
5. J. Ervens, G. Schultz, and R. Seifert, *Biochem. Soc. Trans.*, **19**, 59-63 (1990).
6. H. M. Korchak, K. Vienne, L. E. Rutherford, *et al.*, *Fed. Proc.*, **43**, 2749-2754 (1984).
7. I. Laffafian and M. V. Hallett, *J. Cell Sci.*, **108**, 3199-3205 (1995).
8. H. Lundqvist, M. Gustafsson, A. Johansson, *et al.*, *Biochim. Biophys. Acta*, **1224**, 43-50 (1994).
9. G. M. Omann, R. A. Allen, G. M. Bokoch, *et al.*, *Physiol. Rev.*, **67**, 285-322 (1987).
10. A. D. J. Robertson and J. F. Grutsch, *J. Theor. Biol.*, **125**, 41-60 (1987).
11. F. J. Ross, A. Zimmermann, and H. U. Keller, *J. Cell Sci.*, **88**, 399-406 (1987).
12. T. P. Stossel, *Science*, **260**, 1086-1094 (1993).
13. M. Thelen, B. Dewald, and M. Baggiolini, *Physiol. Rev.*, **73**, 797-812 (1993).