Effect of NMDA on Production of Reactive Oxygen Species by Human Lymphocytes

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Low concentrations (<20 μM) of N-methyl-D-aspartate (NMDA), an agonist of specific receptors of brain glutamatergic systems, promote the formation of reactive oxygen species (ROS) both in the whole blood and in lymphocyte fraction. Further increase in NMDA concentrations led to progressive increase in ROS content in the whole blood, but to its decrease in lymphocyte suspension. The activating effect of NMDA is abolished by antioxidant N-acetylcysteine (5 mM) and NMDA-type glutamate receptor antagonist MK-801 (5 μM). Phorbol myristate acetate (PMA, 1 μM) also increased ROS content in the examined structures. This effect was antagonized by N-acetylcysteine, but not MK-801.

Key Words: *NMDA-receptors; lymphocyte; phorbol myristate acetate; N-acetylcysteine; MK-801*

Functions and properties of glutamate receptors were extensively studied on preparations isolated from the brain of vertebrates, where they mediate excitation and perform various control functions [5,12]. NMDA-type glutamate receptors are responsible for intracellular production of reactive oxygen species (ROS) and excitotoxic effect of glutamate [4,7]. Recently, glutamate receptors were found in the membrane of lymphocytes, where their role is still unknown [1,10]. Our aim was to study ROS production triggered by activation of NMDA receptors of lymphocyte membrane under various conditions.

MATERIALS AND METHODS

The experiments were carried out on heparinized blood (50 U/ml) from healthy donors. Both whole blood diluted 50-fold with physiological saline and lymphocyte suspension isolated from this blood (~3×10^3 cell/ml) were examined. Lymphocytes were isolated as described elsewhere [5]. The aliquots of lymphocytes or whole blood were transferred into incubation medium (physiologic saline) containing luminal (50 μM). Chemiluminescence (cpm) was measured on a Mark III counter (Searle Analytic Inc.). In the presence of luminol, the steady-state level of luminescence in non-activated cells corresponded to the initial rate of ROS production. Activation of blood cells with phorbol myristate acetate (PMA) or NMDA increased luminescence, which reflected the increase in the rate of ROS production. The action of NMDA was compared to the effect of PMA (μM) capable to stimulate ROS production via activation of cytoplasmic oxidases involving protein kinase C [2,9].

RESULTS

Addition of NMDA in increasing concentrations (10-500 μM) to blood suspension dose-dependently increased chemiluminescence (Fig. 1). Since not only lymphocytes, but also other formed elements can produce ROS, further experiments were performed with isolated fraction of lymphocytes.

NMDA in low concentrations (<20 μM) also induced ROS accumulation. But further increase in agonist concentration suppressed this activation and in a concentration of 100 μM this substance produces a inhibitory, but not activating effect on ROS generation (Fig. 1).

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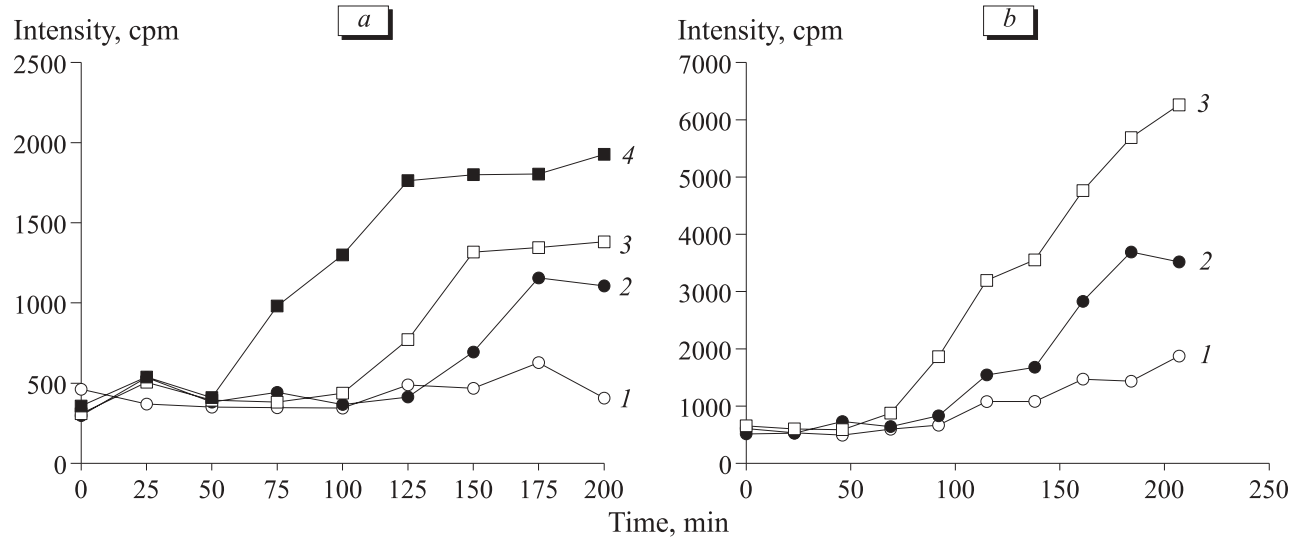

Fig. 1. Effect of NMDA on chemiluminescence of human whole blood. *a*: *1*) control, *2*, *3*, and *4*) NMDA 10, 25, and 50 μM, respectively; *b*: control (*1*), NMDA 0.1 (*2*) and 0.5 mM (*3*).

It can be hypothesized that the whole blood contains factors preventing inhibitory action of high concentrations of NMDA.

The data on the effect of NMDA on lymphocytes obtained for different incubation periods (Table 1) suggest that the effect of this ligand was impeded by MK-801, a specific uncoupler of NMDA receptors with the corresponding ionic channels [8]. It is concluded that NMDA effect of lymphocyte membrane is mediated via a receptor mechanism .

Chemiluminescence recorded in samples containing luminol drastically decreases in the presence of N-acetylcysteine, which regenerates glutathione-dependent antioxidant system and plays a role in antioxidant defense [11,13]. This corroborates the fact that NMDA-induced lymphocyte activation depends on ROS production similarly to analogous process during activation of NMDA-receptors in glutamatergic neurons [3,4,7].

NMDA in a concentration of 10 μM induces a less pronounced burst of ROS production compared to that that observed after addition of 1 μM PMA (Fig. 2). It is noteworthy that activation of cells with PMA is also

TABLE 1. Effect of NMDA on Generation of Reactive Oxygen Species in Lymphocyte Suspension (a Representative Experiment)

Incubation conditions	Incubation, time		
	5	35	50
Control	198	732	1001
+NMDA, 12.5 μM	248	2930	5282
+MK-801, 5 μM	210	317	670

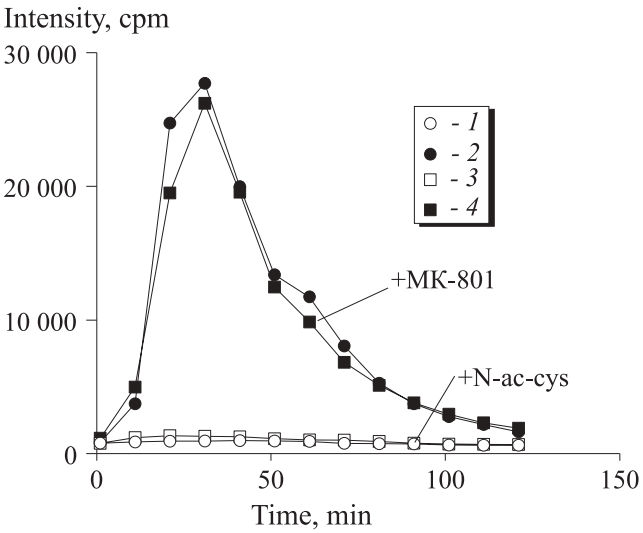

Fig. 2. Effect of PMA on chemiluminescence of lymphocyte suspension. *1*) control (leukocytes+luminol); *2*) PMA (1 μM); *3*) PMA and *N*-acetylcysteine; *4*) PMA and MK-801.

inhibited by N-acetylcysteine, but not MK-801. Therefore, these processes have different nature: the former is mediated by NMDA receptors, while the latter results from direct effects of PMA on cells. It is known that PMA can easily cross the plasmalemma and activate intracellular oxidases, which promote production of superoxide anion [2,9]. The effect of NMDA can be realized with the participation of specific membrane receptors.

It is interesting to compare the dynamics of cell responses to PMA and NMDA. PMA-induced activation was a short-term process, which spontaneously decreased after 30-50 min (Fig. 2). By contrast, the effect of NMDA was less pronounced and developed more slowly. However, there was no tendency to atte-

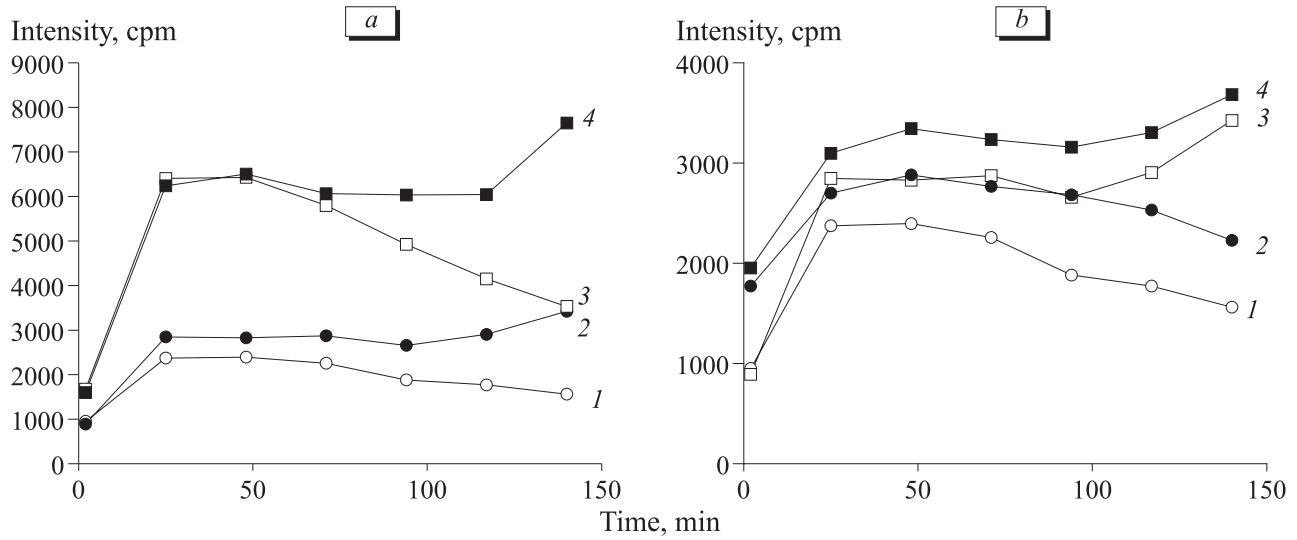

Fig. 3. Blood luminescence of healthy donors and patients with Parkinson's disease in the presence of luminol (50 μM). *a*: *1*) blood of healthy donors, *2*) blood of patients with Parkinson's disease, *3*) blood of healthy donors+PMA (1 μM), *4*) blood of patients+PMA (1 μM); *b*: *1*) blood of healthy donors, *2*) blood of healthy donors+NMDA (10 μM), *3*) blood of patients with Parkinson's disease, *4*) blood of patients+NMDA (10 μM)

nuation of this effect within the same time intervals. At the same time, the addition of PMA to NMDA-activated lymphocytes induced a less pronounced burst of ROS production in comparison with the test, when PMA was added to lymphocytes not activated with NMDA.

Experiments on lymphocytes isolated from the blood of patients with Parkinson's disease (metabolism in this pathology is characterized by enhanced readiness of free radical production) demonstrated enhanced (by several times) steady-state production of ROS. The effects of NMDA and PMA on these lymphocytes were characterized by similar dynamics (Fig. 3). These data attest to possible diagnostic value of the state of NMDA receptors in lymphocytes of patients with neurodegenerative diseases.

Glutamate specifically binds to lymphocyte membrane receptors that are similar to NMDA-type glutamate receptors [1]. Activation of these receptors is accompanied by accumulation of calcium ions in lymphocytes [10]. It can be hypothesized that interaction of NMDA with these receptors activates lymphocytes. Our data corroborate this hypothesis, since cell activation was usually accompanied by intracellular accumulation of ROS.

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REFERENCES

1. I. A. Kostanyan, E. V. Navolotskaya, R. I. Nurieva, *et al.*, *Bioorgan. Khim.*, **23**, 805-808 (1997).
2. B. Allard, E. Long, E. Block, and X. Zhao, *Can. J. Vet. Res.*, **63**, 13-17 (1999).
3. A. Boldyrev, D. Carpenter, M. Huentelman, *et al.*, *Biochim. Biophys. Res. Commun.*, **256**, 320-324 (1999).
4. A. Czyz, G. Baranauskas, and L. Kiedrowski, *J. Neurochem.*, **81**, 379-389 (2002).
5. W. Danysz and Ch. G. Parsons, *Am. Soc. Pharmacol. Exp. Ther.*, **50**, 597-664 (1998).
6. M. H. Goldrosen, P. J. Gannon, M. Lutz, E. D. Holyoke, *J. Immunol. Methods*, **14**, 15-17 (1988).
7. M. Lesort, F. Esclaire, C. Yardin, and J. Hugon, *Neurosci. Lett.*, **221**, 213-216 (1997).
8. D. A. Lowe, H. C. Neijt, and B. Aebischer, *Ibid.*, **113**, 315-321 (1990).
9. H. Lundqvist, P. Follin, L. Khalfan, and C. Dahlgren, *J. Leukoc. Biol.,* **59**, 270-279 (1996).
10. U. Malipiero, C. Heuss, R. Schlpbach, *et al.*, *Eur. J. Immunol.*, **29**, 3053-3062 (1999).
11. C. Pocerhick, M. LaFontaine, and D. A. Butterfield, *Neurochem. Int.*, **36**, 185-191 (2000).
12. I. J. Reynollds and T. G. Hastings, *J. Neurosci.*, **15**, No. 5, 3318-3327 (1995).
13. R. Testa, M. Ghia, F. Mattioli, and S. Borzone, *Fundam. Clin. Pharmacol.*, **12**, 220-224 (1998).