EFFECT OF HYDROCORTISONE ON SUPEROXIDE RADICAL PRODUCTION BY PHAGOCYTIC SPLEEN CELLS

N. N. Vol'skii, V. A. Kozlov, and V. P. Lozovoi

UDC 612.411.017.1:612.262].014.46:615.357.453

KEY WORDS: spleen; superoxide radical; hydrocortisone.

Glucocorticoid hormones have marked immunomodulating properties and are among the most important endogenous regulators of the immune response. For that reason the action of glucocorticoids on migration, proliferation, and functions of lymphocytes and of their separate populations has been well studied [2, 6]. The action of these hormones on the functional state of phagocytic cells (neutrophils and macrophages), which not only are effectors, but also play a leading role in the regulation of immune processes, has received much less study.

The aim of this investigation was to study the effect of hydrocortisone (HC) on production of the superoxide radical (O_2^-), one of the most important metabolic functions of phagocytic cells.

EXPERIMENTAL METHOD

Experiments were carried out on male CBA mice aged 2-4 months, obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR. Hydrocortisone acetate (from Gedeon Richter, Hungary) was injected intraperitoneally into the mice in a dose of 1 mg/mouse in the form of a suspension in medium 199. Control animals were given an intraperitoneal injection of the same volume of medium 199. At fixed times after injection of HC the mice were killed (control and experimental mice simultaneously) and O_2^- production was estimated from the rate of reduction of nitro-blue tetrazolium (nitro-BT), using the method in [8] with minor modifications. For this purpose spleen cells were incubated for 30-45 min at 37°C in medium containing 20 mM phosphate buffer (pH 7.4), 10 mM glucose, 0.2% NaCl, and 0.4 mg/ml of nitro-BT (from Calbiochem, USA). As material for phagocytosis, latex was added to the incubation medium in a concentration of 1 mg/ml. The formazan formed was precipitated by 0.5 N HCl and dissolved in 3 ml of pyridine, after which its optical density was measured at 570 nm on an SF-26 spectrophotometer. The results were expressed in conventional units. A conventional unit was taken to be an increase in optical density at 570 nm by 0.001 optical unit in 45 min.

In some experiments different doses of HC were added in 45 µl of ethyl alcohol to a suspension of splenocytes from intact mice immediately before determination of O_2^- production. To inhibit DNA-dependent RNA synthesis in the splenocytes they were incubated in medium 199 containing 10 µg/ml of actinomycin D (from Serva, West Germany) for 1.5 h at 37°C [4].

To determine the rate of O_2^- production in the presence of an excess of electron donors, various concentrations of NADH (from Reanal, Hungary) were added to the incubation medium. Values of V_{max} and K_m for NADH were determined under these circumstances graphically, using the method of double reciprocals [1]. The experimental data were approximated to linear equations by the method of least squares [5]. The significance of differences was estimated by the Mann–Whitney nonparametric test.

EXPERIMENTAL RESULTS

It will be clear from the results that production of active forms of oxygen by phagocytic spleen cells was increased by 1.8 times only 2 h after addition of HC, it remained high for 2-3 h, and then fell until 12 h after addition, and thereafter remained the same as initially (Fig. 1). A similar time course of O_2^- production also was observed when this parameter was calculated for the spleen as a whole (Fig. 1).

Institute of Clinical Immunology, Siberian Branch, Academy of Medical Sciences of the USSR, Novosibirsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 6, pp. 694-696, June, 1987. Original article submitted October 3, 1986.

 0007-4888/87/0006-0788$12.50

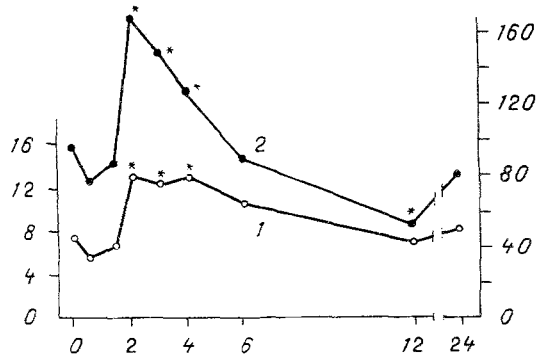

Fig. 1. Superoxide radical production by phagocytic mouse spleen cells at various times after addition of HC. 1) $O_2^{\bullet}$ production in units/10^6 cells; 2) $O_2^{\bullet}$ production in units/spleen. Abscissa, time after addition of HC (in h); ordinate, $O_2^{\bullet}$ production (on the left — in units/10^6 cells, on the right — in units/spleen).

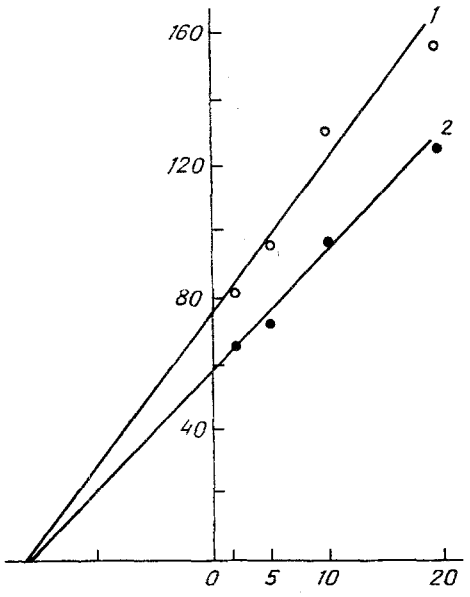

Fig. 2

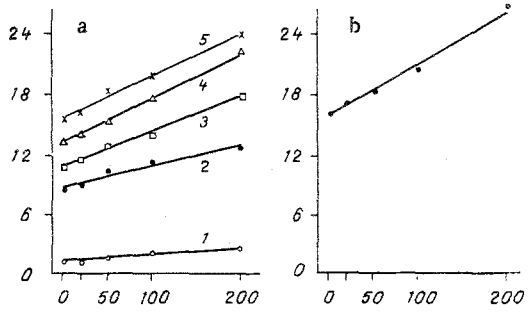

Fig. 3

Fig. 2. Effect of HC on kinetic parameters of NADPH oxidase of phagocytic mouse spleen cells. 1) Control; 2) 3 h after intraperitoneal injection of HC in a dose of 50 mg/kg. Abscissa, (NADH concentration)$^{-1} \cdot 10^3$. Ordinate ($O_2^{\bullet}$ production)$^{-1} \cdot 10^3$.

Fig. 3. Effect of various HC concentrations on rate of superoxide radical production by phagocytic mouse spleen cells. a) Rate of $O_2^{\bullet}$ production with different concentrations of NADH in the incubation medium. 1) Control; 2-5) 50, 100, 200, and 500 μM NADH, respectively; b) maximal rate of $O_2^{\bullet}$ production. Abscissa, HC concentration (in μM). Ordinate, $O_2^{\bullet}$ production (in units/10^6 cells).

The reaction of monovalent reduction of oxygen molecules with the formation of $O_2^{\bullet}$ is catalyzed by the enzyme NADH oxidase, which is located in the plasma membrane of hagocytic cells. It has been shown that under physiological conditions this enzyme utilizes intracellular NADPH as electron donor, but in the case of exogenous addition of reduced pyridine nucleotides, the enzyme can utilize both NADPH and NADH [7]. Since the rate of $O_2^{\bullet}$ production by NADPH oxidase depends both on activity of the enzyme itself (on its amount and its kinetic parameters) and on the level of reduced pyridine nucleotides in the cell, the velocity of this reaction was studied (before and after addition of HC) in the presence of different concentrations of exogenously added NADH. The results of one such experiment are shown in Fig. 2. Clearly, on the

addition of an exogenous electron donor the rate of $O_2^{\bullet}$ production by phagocytic spleen cells increased, in agreement with the Michaelis-Menten kinetics. Under these circumstances the apparent K_m for NADH in a suspension of living spleen cells from CBA mice was 62 ± 12 μM. The value of V_{max} for this reaction was 26.4 ± 9.0 units/10^6 cells. In animals which received 50 mg/kg of HC 3 h before sacrifice, the value of V_{max} was increased by 40-50% without any appreciable changes in the value of K_m for NADH.

To explain the mechanism of action of HC, its effect in vitro was studied in experiments in which HC was added to the incubation medium of a cell suspension taken from intact animals immediately before determination of the rate of $O_2^{\bullet}$ production. It was found that with all concentrations of NADH used (and also in its absence) addition of HC led to an increase in $O_2^{\bullet}$ production, and under these circumstances the reaction velocity rose as a linear function of the HC concentration (Fig. 3). The graph of V_{max} for NADPH oxidase as a function of HC concentration in the incubation medium, plotted from these data (Fig. 3b), is a straight line, i.e., the increase in NADPH oxidase activity was directly proportional to the HC concentration.

Since the effect of HC in these experiments was manifested during incubation (for 30 min), it can be concluded that the lag period of its action does not exceed a few minutes. Such a rapid effect of HC cannot evidently be explained by the classical mechanism of action of steroid hormones, involving reception of the hormone, its transfer into the nucleus, interaction with the genetic apparatus of the cell, and synthesis of specific mRNA and protein, which requires a time measured in hours. Since there is abundant evidence in the literature that glucocorticoid hormones act directly on cell membranes [3], it can be tentatively suggested that in this case also, the effect of HC on $O_2^{\bullet}$ production is effected through its direct action on the plasma membrane of phagocytic cells, activating membrane-bound NADPH oxidase. This hypothesis is supported by the results of the present experiments with actinomycin D, which inhibits DNA-dependent RNA synthesis. If the spleen cells were pre-incubated before the addition of 200 μM HC in medium containing actinomycin D, this had virtually no effect on stimulation of $O_2^{\bullet}$ production by the hormone ($O_2^{\bullet}$ production in the presence of 500 μM NADH was: in the control 21.3 units/10^6 cells, with HC 36.5 units/10^6 cells, and with HC after incubation with actinomycin D 40.6 units/10^6 cells).

Data in the literature [9, 10] on the effect of glucocorticoid hormones on $O_2^{\bullet}$ production by phagocytic cells indicate an inhibitory action of the hormones on this metabolic function. In these investigations, however, the effect could only be recorded a few hours or days after the beginning of action of the hormone. Our results show that one of the earliest actions of glucocorticoids on functional activity of phagocytic cells in the spleen — the principal organ where the humoral immune response takes place — is stimulation of their metabolic activity, and this may be connected with the immunomodulating effect of glucocorticoid hormones.

LITERATURE CITED

1. M. Dixon and E. Webb, Enzymes [Russian translation of Second English Edition], Moscow (1966).
2. E. A. Korneva, V. M. Klimenko, and É. K. Shkhinek, Neurohumoral Basis of Immune Homeostasis [in Russian], Leningrad (1978).
3. P. V. Sergeev, Steroid Hormones [in Russian], Moscow (1984).
4. A. G. Sterlina, I. V. Bogdashin, and B. B. Fuks, Byull. Eksp. Biol. Med. No. 6, 97 (1983).
5. A. I.Tsvetkov, Applied Programs for the Élektronika BZ-21 Microcomputer [in Russian], Moscow (1982).
6. V. F. Chebotarev, Endocrine Regulation of Immunogenesis [in Russian], Kiev (1979).
7. R. C. Allen, S. J. Yevich, R. W. Orth, et al., Biochem. Biophys. Res. Commun., 60, No. 3, 909 (1974).
8. R. L. Baehner and D. G. Nathan, N. Engl. J. Med., 278, No. 4, 971 (1968).
9. O. Clemmensen, V. Andersen, N. E. Hansen, et al., Acta Med. Scand., 199, No. 1, 105 (1976).
10. D. R. Miller and H. G. Kaplan, Pediatrics, 45, No. 3, 861 (1970).