LITERATURE CITED

1. B. V. Aleshin, Histophysiology of the Hypothalamo-Hypophyseal System [in Russian], Moscow (1971).
2. V. I. Gubskii, Byull. Éksp. Biol. Med., No. 4, 54 (1964).
3. I. A. Krasnovskaya and V. V. Kuzik, Arkh. Anat., No. 8, 38 (1985).
4. I.A. Krasnovskaya, T. V. Sheibak, and A. L. Polenov, Byull. Éksp. Biol. Med., No. 2, 666 (1987).
5. A. L. Polenov, Arkh. Anat., No. 9, 3 (1962).
6. A. L. Polenov, Hypothalamic Neurosecretion [in Russian], Leningrad (1968).
7. A. L. Polenov, Usp. Fiziol. Nauk, 10, No. 1, 28 (1979).
8. B. Ahrén, H. I. Bergtsson, and P. Hedner, Endocrinology, 119, 1058 (1986).
9. T. Balla, P. Enyedi, A. Spät, and F. A. Antoni, Endocrinology, 117, 421 (1985).
10. J. Garcia, G. W. Harris, and W. J. Schindler, J. Physiol. (London), 170, 487 (1964).
11. D. S. Gross, Endocrinology, 112, 733 (1983).
12. R. Kvetnansky, F. J. H. Tilders, I. D. Zoest, et al., Neuroendocrinology, 45, 318 (1987).
13. H. Lipscomb, D. Hathaway, and D. Gard, Clin. Res., 9, 29 (1961).
14. R. Meidan and A. J. Hsueh, Endocrinology, 116, 416 (1985).
15. N. Philip and E. F. Grollman, FEBS Lett., 202, 193 (1986).
16. W. Vale, C. Rivier, and M. Grown, Handbook of the Hypothalamus, New York (1980).
17. W. Wetsel and J. D. Fernström, Brain Res., 370, 315 (1986).

ROLE OF INHIBITION OF CELL Na^+/H^+ EXCHANGE AND GLYCOLYSIS IN ANTIVIRAL ACTION

V. A. Kirillov, L. N. Goreva, and V. I. Votyakov

UDC 578.245.083.226.04:615.218:578

KEY WORDS: Na^+/H^+ exchange; lactic acid; acification; remantadine

Antiviral agents include compounds with the ability to change the mechanism of interaction between virus and cell that has become established in evolution [3]. The antiviral action of substances can be realized through action on the structural and functional state of cells, both before and after infection. Inhibition of the early stages of infection is most promising, for in this case the development of pathological processes in the cell is prevented. We know that many viruses penetrate into the cytoplasm by viropexis (a variant of receptor endocytosis) – a complex physiological mechanism developed by the cell in the course of evolution in order to seize macromolecules [1, 14]. To realize the next stage, namely uncoating of the virus, a considerable fall of the intraendosomal pH is required [10, 13]. The writers previously discovered the nature of acidification of the internal medium of virus-containing endosomes, effected by membrane-bound systems of the cell [7, 8]. As a tool to act upon these systems we used remantadine, which has a prophylactic and early therapeutic action [10] and possesses membranotropic properties [9]. By this approach it is possible not only to discover the mechanism of the anti-influenzal action of remantadine at the stage of uncoating of the virus, but also to determine to what degree suppression of processes responsible for reduction of the intraendosomal pH is coupled with inhibition of viral activity.

We therefore decided to study the effect of remantadine, in different doses and the time of preliminary treatment of the cells on the process of acidification of the incubation medium of chick embryonic fibroblasts.

EXPERIMENTAL METHOD

Cell suspensions of chick embryonic fibroblasts obtained by removal of a monolayer culture from the substrate by treatment with 0.1% trypsin solution for 30 sec were used in the experiments. Changes of pH in the cell suspension were recorded potentiometrically [7] at 25°C in medium of the following composition: 0.15 M NaCl, 1 mM HEPES/KOH (pH 7.4) with the addition of either KCl (10 mM) or glucose (1%); 0.25 ml of a thick cell suspen-

Belorussian Research Institute of Epidermiology and Microbiology, Minsk. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 33-35, January, 1990. Original article submitted May 15, 1989.

 0007-4888/90/0001-0040$12.50

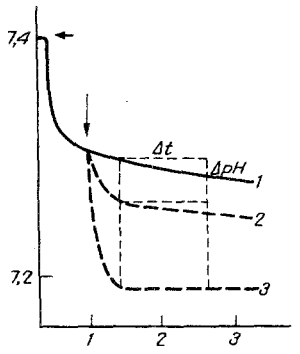

Fig. 1

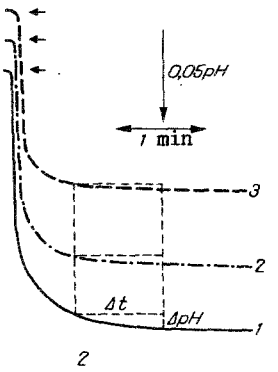

Fig. 2

Fig. 1. Curves of acidification of incubation medium by chick embryonic fibroblasts on the addition of remantadine. 1) Control; 2, 3) addition of remantadine to cells in concentration of 50 and 500 μg/ml, respectively. Abscissa, time (in min); ordinate, pH of medium. Bold arrow indicates time of addition to cell suspension to cuvette, thin arrow — time of addition of remandatine.

Fig. 2. Curves of acidification of incubation medium by chick embryonic fibroblasts, treated beforehand with remantadine in concentration of 50 μg/ml. duration of preliminary treatment: 1, 2, 3) 0 (control), 10, and 30 min, respectively. Arrow indicates time of addition of sample to cuvette.

sion (concentration $3 \cdot 10^7$ cells/ml) was added to 4.65 ml of medium. After 1 min, 90.1 ml of remantadine, diluted in physiological saline to a certain concentration allowing for the volume of medium in the cuvette, was added. For preliminary treatment 0.25 ml of the cell suspension was incubated with remantadine in a concentration of 50 μg/ml at 25°C for various times ranging from 0 to 30 min, after which the mixture was added to the cuvette where besides medium of the composition described above, remantadine was also present in a concentration of 50 μg/ml. In all cases, instead of remantadine, an equal volume of physiological saline was added to the control samples.

EXPERIMENTAL RESULTS

The general appearance of the acidification curve of the incubation medium of the chick embryonic fibroblasts is shown in Fig. 1. As we demonstrated previously, the nature of this process depends on the energy metabolism of the cell [7]. For instance, acidification of the medium by cells in the presence of exogenous glycolysis, namely of proton translocation by a Na^+/H^+ exchange mechanism.

On addition to cells, remantadine causes an initial sharp fall in pH of the medium (Fig. 1). This is associated with the chemical properties of remantadine itself, which is the salt of a weak base and a strong acid. This is confirmed by the fact that, if added in the same concentration to a cell-free system, remantadine causes a similar fall of pH of the solution in the cuvette. After a short time interval, of the order of 10-20 sec (depending on the concentration of remantadine added), the fast phase is replaced by a slow phase, due to release of acid products directly from the cytoplasm. Accordingly, the basic parameter for analysis of the kinetics of the acidification process was the rate of acidification in the slow phase, defined as the increase in the H^+ concentration (Δ pH) in unit time Δt).

As Fig. 1 and Table 1 show, on addition to the cells remantadine caused a decrease in the rate of acidification with effect from the first few seconds of the slow phase. The inhibitory effect increased with an increase in the concentration of the preparation. For instance, in a concentration of 50 μg/ml, which corresponds to the maximal tolerated dose for chick embryonic fibroblasts [4], remantadine reduced the rate of acidification by only 10%, whereas in a concentration of 500 μg/ml, known to be toxic, remantadine completely suppressed the acidification process. Further investigation showed that the degree of in-

TABLE 1. Dependence of Rate of Acidification of Incubation Medium by Chick Embryonic Fibroblasts of Remantadine Concentration (M ± m)

Concentration of remantadine, μg/ml	Rate of acidification of medium, % of control	
	in absence of glucose	in presence of glucose
25	100	100
50	89,0±1,9	93,0±1,3
125	63,0±2,5	66,0±2,0
250	25,0±2,4	26,0±2,8
500	0	0

TABLE 2. Dependence of Rate of Acidification of Incubation Medium by Chick Embryonic Fibroblasts on Duration of Preliminary Treatment of Cells with Remantadine in a Concentration of 50 μg/ml (M ± m)

Duration of preliminary treatment, min	Rate of acidification of medium, % of control	
	in absence of glucose	in presence of glucose
5	73,0±2,5	75,0±2,6
10	55,0±3,9	60,0±3,4
20	43,0±3,5	48,0±3,7
30	33,0±3,7	40,0±3,2

hibition of the acidification process increases with an increase in the duration of preliminary treatment of the cells with remantadine (Fig. 2; Table 2). In particular, preliminary treatment of the cells with remantadine in a concentration of 50 μg/ml for 10 min reduced the rate of acidification by 40-45%, whereas after treatment for 30 min the effect was increased by up to 60-65%.

The lowering of the rate of acidification which we found can be explained as follows. On addition to the cells, because of its lipophilicity, remantadine is built into the protein-lipid membrane [12]. This leads to modification of the physical state of the plasmalemma [11], the probable result of which is a change in transport activity of the glucose carrier and the Na^+/H^+ exchanger. The final result of the chain of events described is a fall of the level of lactic acid formation as a result of a deficit of the substrate for glycolysis and inhibition of function of the Na^+/H^+ exchanger, which leads to a decrease in the qualtity of acid products entering the external medium. Data indicating no difference in the effect of the remantadine-dependent fall of the rate of acidification, taking place both through a mechanism of Na^+/H^+ exchange (in the absence of glucose) and through release of the end product glycolysis, namely lactic acid (in the presence of glucose), are evidence of the nonspecific character of the inhibitory action of remantadine in the concentrations used on the acidification process (Tables 1 and 2).

As we showed previously on chick embryonic fibroblasts infected with influenza virus, the processes of acidification described above are utilized by the virus at the uncoating stage, i.e., the lowering of the pH in virus-containing endosomes takes place as a result of translocation of H^+ through a mechanism of Na^+/H^+ exchange and on account of diffusion of lactic acid [8]. For that reason remantadine, just as in the case of cells, evidently induces a fall in the rate of acidification of the contents of the virus-induced a fall in the rate of acidification of the contents of the virus-induced endosomes, the inner space of which, it must be noted, is external relative to the cytoplasm. Such a fall in the rate of acidification of the contents of the virus-induced endosomes, the inner space of which, it must be noted, is external relative to the cytoplasm. Such a fall in the rate of acidification probably leads to an increase in the time required for the value of the intraendosomal pH to reach the critical level for uncoating the influenza virus, and virus which has not succeeded in uncoating in this way will be supplied to the endosome, which is a transport organelle, to the lysosome, on entry into which it will be degraded [5, 6]. In other words, because of the fall in the rate of acidification of the internal medium of the endosome under the influence of remantadine, the virus particle holds over the time allocated by it for activation of its genome, and it will be utilized by the cellular lysosome (the noninfectious path), which will lead to inhibition of infection as a whole.

Thus, the results of these investigations indicate that the inhibitory effect of remanta dine on uncoating of the influenza virus is realized through prevention of the fall of the intraendosomal pH to the critical level on account of reduction in the quantity of acid products transported inside the virus-containing endosomes in the form of H^+ and lactic acid, as a result of inhibition of cellular Na^+/H^+ exchange and glycolysis.

LITERATURE CITED

1. V. I. Bubovich, G. M. Ryazantseva, and M. K. Indulen, Early Stages of Multiplication of Microviruses and the Effect of Inhibitors on Them [in Russian], Riga (1987).

2. A. G. Bukrinskaya and N. K. Sharova, Vorp. Virusol., No. 3, 260 (1984).
3. V. I. Votyakov, Molecular Biology of Viruses, Chemotherapy and Chemoprophylaxis of Virus Infections [in Russian], Minsk (1974), pp. 10-28.
4. V. I. Votyakov, E. I. Boreko, and L. K. Zaporozhets, Vorp. Virusol., No. 4, 468 (1984).
5. R. N. Glebov, Endocytosis and Exocytosis [in Russian], Moscow (1987).
6. R. Dean, Breakdown Processes in the Cell [Russian translation], Moscow (1981).
7. V. A. Kirillov, Yu. M. Sudnik, I. A. Podol'skaya, and V. I. Votyakov, Dokl. Akad. Nauk SSSR, 295, No. 4, 998 (1987).
8. V. A. Kirillov, Yu. M. Sudnik, L. N. Goreva, and V. I. Votyakov, Structural Dynamics of Biological Membranes and Its Role in the Regulation of Photobiological and Receptor Processes [in Russian], Minsk (1988), p. 58.
9. V. A. Kirillov, A. N. Rudenok, L. N. Goreva, et al., Vest. Akad. Nauk Belor. SSR, Ser. Biol. Nauk, No. 1, 84 (1988).
10. Yu. M. Sudnik, V. A. Kirillov, Yu. E. Klinger, et al., Vest. Akad. Nauk Belor. SSR, Ser. Biol. Nauk, No. 2, 53 (1984).
11. I. G. Kharitonenkov, Remantadine and other Inhibitors of Viruses [in Russian], Riga (1982), pp. 105-121.
12. M.K. Jain, We N. Yen-Min, T. K. Morgan, et al., Chem. Phys. Lipids, 17, 71 (1976).
13. J. Lenard and D. K. Miller, Cell, 28, 5 (1982).
14. M. Marsh, Biochem. J., 218, 1 (1984).

ACTIVATION OF ENDOGENOUS LIPID PEROXIDATION IN THE BRAIN DURING OXIDATIVE STRESS INDUCED BY IRON AND ITS PREVENTION BY VITAMIN E

G. M. Kainova, D. Markovska,
D. Staneva, and V. E. Kagan

UDC 616.831-008.939.15-39-02:615.31:546.72]-085.356:577.161.3]-039.71

KEY WORDS: lipid peroxidation; stress; vitamin E

The key functions of nerve cells, namely creation and maintenance of the transmembrane potential, reception and subsequent transmission of the signal, production and regulatory action of secondary messengers, and uptake and release of neurotransmitters, are highly sensitive to excessive accumulation of endogenous products of lipid peroxidation (LPO) in membrane structures [1-4]. Accordingly LPO is regarded nowadays as an etiologic or pathogenetic factor in several diseases of the CN (Parkinson's disease, Down's syndrome, schizophrenia, epilepsy, etc.), and also in complications caused by administration of neuroleptics and antidepressants [5, 6]. The role of activation of free-radical reactions and of LPO in particular, in the process of brain aging, accompanied by depression of activity of enzymic and nonenzymic antioxidative systems, also has been discussed [7-9]. The most important of the latter in the membrane structures of the brain is vitamin E, a lipid-soluble interceptor of free radicals, the amount of which falls much more slowly in animals kept on a vitamin E-deficient diet than in peripheral tissues [7-11].

Administration of LPO inducers to animals causes the development of oxidative stress and rapid exhaustion of vitamin E reserves in peripheral tissues, although membrane structures of the brain are much more resistant and under the same conditions activation of LPO and depression of the vitamin E level cannot be recorded in them [7, 12]. The obtaining of an effect of vitamin E-dependent activation of endogenous LPO in the membrane structures of the brain in vivo is interesting in connection with the formation of a model of premature brain aging.

This paper describes an attempt to induce oxidative stress in the rat brain by repeated injections of iron into the animals, the standard substance used as a catalyst of breakdown of lipid hydroperoxides with the formation of radicals in vitro, and to estimate the sensitivity of the developing endogenous LPO to exogenous vitamin E. In view of data showing the

Institute of Physiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 109, No. 1, pp. 35-37, January, 1990. Original article submitted June 26, 1989.

