DAMAGE TO RAT BRAIN SYNAPTOSOMAL CREATINE PHOSPHOKINASE THROUGH ACTIVATION OF MOLECULAR OXYGEN

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UDC 616.831-008.931:577.152.273]-02:
616.831-008.922.1-008.61

KEY WORDS: creatine phosphokinase; synaptosomes; rat brain; activation of molecular oxygen

Activation of molecular oxygen is an essential factor in the modification of cellular structures. The process of this kind that has been studied the most is modification of biological membranes through activation of lipid peroxidation (LPO) [2, 12]. It must be recalled, however, that activation of molecular oxygen is not always the result of membrane processes such as, for example, the functioning of electron-transport chains, cytochrome P-450, monoamine oxidase, etc. [3, 4]. In some cases molecular oxygen can also be activated in the cytoplasm, for example, during oxidation of substrates by xanthine and aldehyde oxidases, by self-oxidation of low-molecular-weight compounds and, in particular, catecholamines, and through interaction with metals of variable valency [2, 10, 11]. Not only lipids of biological membranes, but also soluble proteins can naturally be the targets for oxygen activated in this way. As an example may be mentioned the auto-inactivation of xanthine oxidase during oxidation of xanthine [10]. It is therefore logical to expect that activation of molecular oxygen may be the cause of disturbance of enzyme activity of cytosol proteins. The aim of the present investigation was accordingly to study the effect of activation of oxygen on activity of one typical cytosol enzyme, creatine phosphokinase (CPK), in rat brain synaptosomes.

EXPERIMENTAL METHOD

Synaptosomes were isolated from the gray matter of the rat brain [8]. The concentration of synaptosomal protein was determined by the method in [6] and CPK activity by the method in [9].

Molecular oxygen was activated by addition of Fe^{++} and ascorbate ions to a suspension of synaptosomes. Activation of LPO was judged by accumulation of malonic dialdehyde (MDA). The MDA concentration was determined as the formation of a colored complex with 2-thiobarbituric acid [2].

The sucrose, D-glucose, dithiothreitol, ascorbate, HEPES, 2-thiobarbituric acid, and α-tocopherol used in the work were obtained from "Serva" (West Germany), hexokinase and glucose-6-phosphate dehydrogenase from "Sigma" (USA), and AMP, ADP, and creatine phosphate from "Reanal" (Hungary). The remaining reagents were of Soviet origin and of the chemically pure grade.

EXPERIMENTAL RESULTS

As was pointed out above, activation of molecular oxygen in the cell can take place in several ways, including during its reduction with the aid of ions of metals of variable valency. Accordingly, in the first series of experiments the effect of an Fe^{++}/ascorbate system on CPK activity and on the MDA concentration in rat brain synaptosomes was investigated. As will be clear from the results in Table 1, during incubation of the synaptosomes with Fe^{++}/ascorbate, CPK activity was reduced and LPO products accumulated. Lowering of CPK activity through the action of Fe^{++}/ascorbate was not accompanied by any change in the value of K_m of the enzyme (K_m = 1.0-2.0 mM for creatine phosphate). The most likely explanation

All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, I. P. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 6, pp. 680-682, June, 1989. Original article submitted July 8, 1988.

TABLE 1. Changes in MDA Concentration and CPK Activity in Rat Brain Synaptosomes under the Influence of Fe^{++}/Ascorbate (M ± m; n = 6)

Parameter	Control, %	Fe^{++} (1 μM), ascorbate ($15\ \mu M$)$_1$	Fe^{++} (3 μM), ascorbate ($14\ \mu M$)$_2$
MDA concentration	100	489±200*	623±286*
CPK activity	100	57,8±8,0*	43,0±9,0*

Legend. MDA concentration and CPK activity determined after incubation of synaptosomes (100 μg protein/ml) with Fe^{++}/ascorbate for 30 min at 37°C. CPK activity and MDA concentrations expressed in % of control. $r_1 = -0.88$, $r_2 = -0.64$, $*p < 0.05$.

is that during the action of Fe^{++}/ascorbate a decrease in activity took place, not because of a decrease in specific activity of all CPK molecules, but as a result of inactivation of some molecules of the enzyme.

It is important to note that with an increase in concentration of Fe^{++}/ascorbate from 1/15 to 3/45 μM, besides intensification of LPO, CPK activity also was reduced. This indicates a connection between reduction of CPK activity and MDA accumulation that characterizes LPO, and it is confirmed by the high values of the coefficients of correlation between these parameters (Table 1). The character of this connection may be twofold. On the one hand, this correlation may reflect a direct connection between activation of LPO and damage to CPK; on the other hand, correlation of this kind is possible if both LPO activation and CPK damage take place independently, but with the participation of the same damaging factor, i.e., of active forms of oxygen. Support for this hypothesis is given by the decrease in the coefficient of correlation with an increase in concentration of Fe^{++}/ascorbate. Such a decrease can be explained on the grounds that in the presence of high concentrations, ascorbate has an inhibitory action on LPO [5].

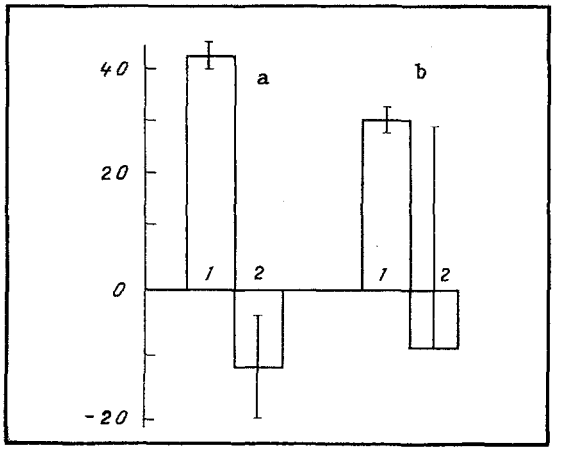

Fig. 1. Effect of α-tocopherol (10^{-4} mole/mg protein) on MDA accumulation and CPK activity in rat brain synaptosomes under the influence of Fe^{++}/ascorbate. a) Fe^{++} 1 μM, ascorbate 15 μM; b) Fe^{++} 3 μM, ascorbate 45 μM. Effect of tocopherol represented as % of inhibition of LPO and change in CPK activity relative to values obtained under identical conditions but in the absence of α-tocopherol. 1) Inhibition of LPO, 2) change in CPK activity.

The character of the connection between LPO and CPK damage can be explained by the use of selective inhibition of one of these processes. For this purpose, in the next series of experiments we studied the effect of the natural lipophilic antioxidant α-tocopherol on MDA accumulation and CPK activity under the influence of Fe^{++}/ascorbate. It will be clear from the data in Fig. 1 that α-tocopherol inhibits LPO but does not lower the level of damage to CPK. It can accordingly be concluded that damage to CPK is caused by the direct action of active forms of oxygen, generated by the Fe^{++}/ascorbate system, and not by activation of LPO. The results are in agreement with data indicating that damage to some proteins on activation of molecular oxygen is not connected with LPO activation [7, 13]. This conclusion is sufficiently important because activation of molecular oxygen, in the modern view [2, 12], is a key mechanism in the etiology and pathogenesis of several diseases. An intensive search for effective antioxidants is therefore currently in progress. However, most attention has been paid to the ability of these compounds to inhibit LPO of cell membranes. The results of the present investigation, and also those obtained in other studies, are evidence that activation of molecular oxygen can lead to damage to enzymes and DNA [1, 13]. Accordingly, to develop effective methods of correcting pathological states associated with a role of active oxygen, it is advisable to use not only LPO inhibitors, but also compounds protecting the enzymic and genetic apparatus of the cell against the action forms of oxygen.

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