Lipid Peroxidation in the Depolarization of Synaptosomes

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One of the important elements in the pathogenesis of epilepsy is disturbed regulation of lipid peroxidation (LPO) in the central nervous system (CNS), leading to the uncompensated activation of LPO [2,3]. The mechanism of the occurrence of LPO imbalance in epileptogenesis is too complicated to be studied *in vivo*, and simpler, model experiments are required for its investigation. As one of the main electrophysiological features of the "epileptic neuron" is a stable depolarization of its membrane, or what is known as the paroxysmal depolarization shift, one of the suitable models in this regard may be depolarized synaptosomes.

In this connection we carried out a study of LPO activity in synaptosomes resulting from different methods of depolarization.

MATERIALS AND METHODS

Synaptosomes were obtained after Hajös [8] from the cerebral cortex of Wistar rats weighing 180-220 g. The incubation medium contained 104 mM NaCl, 5 mM KCl, 1.2 mM NaH_2PO_4, 0.75 mM $CaCl_2$, 1.3 mM $MgCl_2$, 10 mM glucose, and 20 mM Tris-HCl, pH 7.4 (37°C). The final protein content in the samples, measured after Lowry [10], was 1-3 mg/ml.

Depolarization of synaptosomes was performed by three methods: 1) electrostimulation of a synaptosome suspension [6]; 2) increase of K^+ in the incubation medium up to 33 mM, and 3) inhibition of Na,K-ATPase of synapse membranes by ouabain (1 mM). Electrostimulation of the synaptosome suspension was performed using 4-mm-wide platinum electrodes placed at 12-mm intervals. An ESU-2 multipurpose electrostimulator served as the source of rectangular pulses. The characteristics of the pulses were monitored using a VC-9 oscillograph (Nihon Kohden, Japan).

Taking into account the reliable thermoactivation of LPO reactions in synaptosomes, observed during transfer from 4°C to 37°C [4], the depolarization influence was started 20 min after preincubation of synaptosomes at 37°C. The duration of the depolarization treatment was 10 min in the case of electrostimulation and 20 min when K^+ or ouabain was added.

The activity of LPO in the synaptosomes was judged by the level of 2-thiobarbituric acid-reacting products (TBRP) in the suspension. TBRP were determined as described earlier [4] on an Aminco-Chance spectrophotometer (USA).

All chemicals used were manufactured by Serva and Merck.

RESULTS

It was shown that a 10-min stimulation of synaptosomes with electric pulses of 50 pairs/sec (50 Hz) counterpolar rectangular pulses with an amplitude of 10 V failed to change the TBRP concentration in the

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TABLE 1. Effect of Synaptosome Depolarization on the Level of TBRP ($M \pm m$)

Depolarization stimulus	TBRP level
Electrostimulation	
10 V, 50 Hz	110±10 (3)
20 V, 50 Hz	200±18* (3)
40 V, 50 Hz	283±20* (3)
30 V, 125 Hz	410±20* (3)
KCl (33 mM)	137±4** (9)
Ouabain (1 mM)	124±3** (30)
Ouabain (1 mM) + tocopherol (0.6 mM)	108±3 (9)

Note. The TBRP level is expressed as a percentage of the control level (100%). * : $p<0.01$; ** : $p<0.05$ in comparison with control. The number of repeated experiments is shown in parentheses.

suspension (Table 1). An increase of the amplitude to 20 and 40 V induced a dramatic (respectively, twofold and threefold) rise of the TBRP level in the synaptosomes, reflecting an activation of LPO processes. An increase of the electrostimulation frequency from 50 Hz to 125 Hz with an amplitude of 30 V also led to a 4-fold rise of the TBRP level in the synaptosomes (Table 1). Thus, the electrostimulation-induced depolarization of synaptosomes was accompanied by an activation of LPO processes.

Similarly to electrostimulation, two other methods of depolarization of isolated nerve endings (increase of the K^+ concentration in the medium and ouabain-induced inhibition of Na/K-ATPase) also led to a TBRP increase in the synaptosomes by 15-30% (Table 1). The addition to the synaptosomes of the antioxidant α - tocopherol (0.6 mM) simultaneously with ouabain prevented the rise of TBRP in the suspension.

Thus, three different approaches to the depolarization of isolated nerve endings uniformly led to the activation of LPO processes in these. Quite likely, the observed effect is caused by the activation of endogenous phospholipases during synaptosome depolarization, specifically type A phospholipases, as has been noted by other workers [5,7,9]. Earlier we showed that the activation of endogenous

phospholipase-produced hydrolysis in synaptosomes intensifies LPO processes [1]. This validates the earlier inference [4] that one of the mechanisms of LPO activation in epileptogenesis is type A phospholipase activation in the region of hyperactivity.

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