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Phosphorylation of Nuclear Proteins in a Chronic, Uncontrollable Stress Model

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VELICHKOVA, A. A. *Phosphorylation of nuclear proteins in a chronic, uncontrollable stress model.* PHARMACOL BIOCHEM BEHAV 51(2/3) 231-233, 1995.—The changes in the phosphorylation of nuclear proteins isolated from cell nuclei of hypothalamus, cerebral cortex, and hippocampus of rats subjected for varying times to a chronic uncontrollable stress model were investigated. A brief duration (24 h) induced a substantial increase of the phosphorylation of nuclear proteins isolated from hypothalamus (270%), from the cerebral cortex (ca. 230%), and from the hippocampus (ca. 160%). More extended durations (96 and 168 h) were accompanied by a statistically significant decrease in the degree of phosphorylation of proteins.

Phosphorylation Nuclear proteins Quercetin

THE PHOSPHORYLATION of protein molecules is a mechanism responsible for the posttranslational regulation of their functions. This process leads to changes in the activities of many enzymes and receptors; it changes the permeability of the ionic channels and influences gene expression. Protein phosphorylation is accompanied by dephosphorylation processes through which the original structure and activity of the protein molecules are restored. The rate of the processes in both directions is very high, which also determines their significance for the physiologic response of the cell (2,3,10). Protein phosphorylation is substantially influenced by the concentration of the second messengers (1).

Changes in protein phosphorylation are observed when the functional state of the organism is changed—for example, as a result of hunger or intensive motor loading (14,15,17). Because many enzymes participate in the processes of protein phosphorylation, different inhibitors, including quercetin, are used to determine the action of various protein kinases. Quercetin inhibits cAMP-independent protein kinases, protein kinase C, the transfer of calcium ions, and protein biosynthesis (9,13). However, it has no effect on the activity of cAMP-dependent protein kinase (13).

Chronic, uncontrollable stress is produce by REM-sleep deprivation, which causes changes in some physiologic, endocrine, and neurochemical values (7,11).

The aim of the present study was to determine what changes take place in the phosphorylation of nuclear proteins when a chronic, uncontrollable stress model is used, as well to identify the probable factors responsible for the processes.

METHOD

The experiments were carried out on Wistar rats weighing 180–200 g. The rats were randomized into four groups according to on the experimental conditions: a) 24, b) 96, and c) 168 h exposure, and d) control. The experimental groups contained six rats each. The animals were bred in an air-conditioned room with an ambient temperature of 24°C and on a 14 L : 10 D cycle (lights on at 0700 h). The experimental animals were placed in cages containing a 5-cm-thick water layer. The phosphorylation of nuclear proteins was carried out after uncontrollable stress lasting 24, 96, and 168 h, because previous investigations demonstrated that these periods are associated with critical changes in the adaptive capabilities of the CNS (7,11).

Preparation of the Nuclei

The rats were decapitated and their brains were immediately removed, then washed in 0.32 M sucrose; the following structures were separated in the cold: hypothalamus, cerebral cortex, and hippocampus. The tissue was homogenized in 1.6 M sucrose (10 mM $MgCl_2$, 10 mM Tris HCl, pH 7.4) and the cell nuclei were sedimented by centrifugation for 1 h at 22,000 × *g*, using a Janetzki K 24 centrifuge [(15) Dresden, Germany]. The phosphorylation of proteins was studied by incubating the nuclei in the following mixture: 0.32 M sucrose, 1 mM $MgCl_2$, 50 mM KCl, 50 mM NaCl, 5 mM $Na_2S_2O_5$, 2 mM 2-mercaptoethanol, 50 mM Tris HCl, pH 7.4, 10 mM ATP, and ^{32}P-NaH_2PO_4 (5 μCi/50 μg DNA) [(10), with some modi-

fications]. Incubation was carried out with or without quercetin for 5 min at 37°C. The quercetin was added in the incubation medium at a concentration of 0.15 mM. The reaction was terminated by spotting aliquots of the reaction mixture (20 μl) onto Whatman CF/A filter paper (Whatman International Ltd, Maidstone, England) followed by precipitation in 10% Cl_3CCOOH containing 1% $Na_4P_2O_7$. After extensive washing with 5% Cl_3CCOOH containing 1% $Na_4P_2O_7$, the papers were washed with ethanol and dried in diethylether before determination of the radioactivity in a Beckman LS 3801 liquid-scintillation system (Fullerton, CA).

Protein was determined quantitatively after (8). The results presented are the averaged data of six observations from three experiments, and were evaluated using the Mann-Whitney test.

RESULTS

The chronic, uncontrolled stress model used caused changes in the phosphorylation of the total nuclear proteins isolated from the hypothalamus, cerebral cortex, and hippocampus. The observed changes depended on the duration of the experiment (Fig. 1). A brief exposure (24 h) induced a substantial increase in phosphorylation of the nucler proteins isolated from the hypothalamus (270%), cerebral cortex (ca. 230%), and hippocampus (ca. 160%).

The effect of quercetin was demonstrated by an decrease in the protein kinase activity of the control animals by about 43% in the hippocampus and about 54% in the cerebral cortex (Figs. 2 and 3). After a short exposure (24 h), there was an approximately 20% increase in protein phosphorylation in the presence of the inhibitor, in both the hippocampus and cerebral cortex. The prolongation of the state of stress induced an increase in the phosphorylation of the nuclear proteins isolated from the hippocampus by another 10%, whereas no substantial changes were observed in proteins isolated from the cerebral cortex.

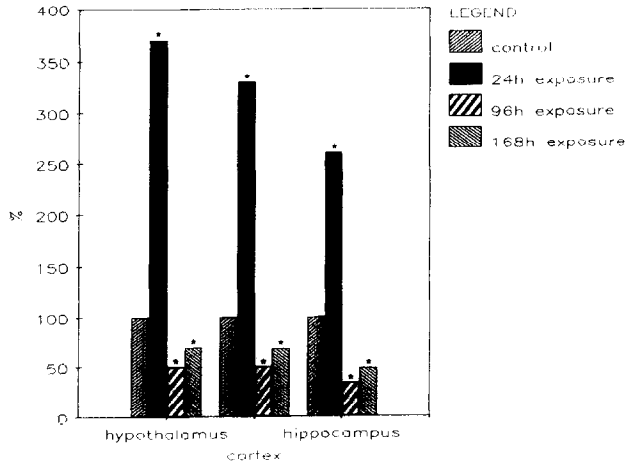

FIG. 1. Changes in phosphorylation of nuclear proteins isolated from the hypothalamus, cerebral cortex, and hippocampus of rats subjected to chronic, uncontrollable stress model for 24, 96, and 168 h exposure. Data represent percent changes from control values. *Significantly different, $p < 0.05$.

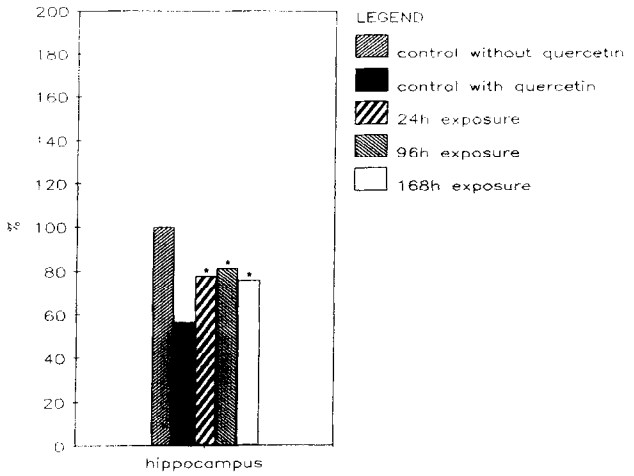

FIG. 2. Effect of quercetin on the phosphorylation of nuclear proteins isolated from hippocampus after 24, 96, and 168 h exposure. Data represent mean percent changes from control values. *Significantly different, $p < 0.05$.

DISCUSSION

Our data suggest a definite regional dependence in the brain on nuclear protein phosphorylation, which reflects a specificity of the functions and metabolic processes taking place in the structures studied. It should be pointed out that in experimental animals, the highest protein phosphorylation values were found in the hypothalamus, whereas in the control animals the highest phosphorylation level was obtained in the cerebral cortex (18). The data are in agreement with the sharp increase in the phosphorylation of membrane proteins isolated from the brain of animals subjected to intensive motor loading (16,17). The experimental duration (96 and 168 h) was accompanied by a statistically significant decrease of the degree of phosphorylation of the nuclear proteins, compared with the

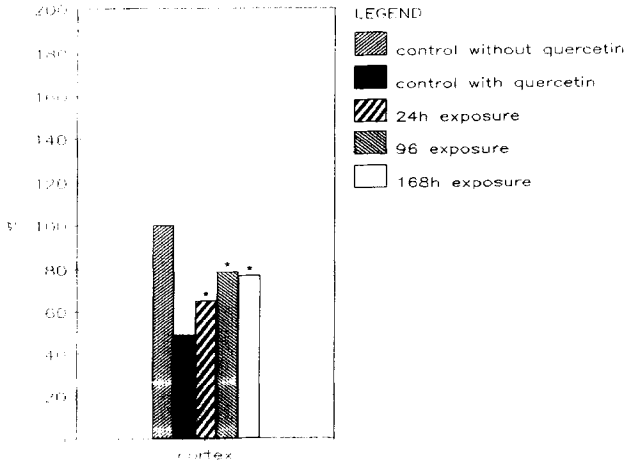

FIG. 3. Effect of quercetin on the phosphorylation of nuclear proteins isolated from cerebral cortex after 24, 96, and 168 h exposure. Data represent mean percent changes from control values. *Significantly different, $p < 0.05$.

control values. Similar changes were also observed in the hunger model, but in the uncontrollable stress model the phosphorylation we observed was much more pronounced (14).

The observed changes in the phosphorylation of nuclear proteins in the presence of quercetin are probably due to the increased activity of the cAMP-dependent protein kinase, resulting from higher cAMP concentrations in the nuclei caused by the changed external conditions. On the other hand, cAMP stimulates the phosphorylation of the protein phosphatase inhibitor I-1 (4,19). The phosphorylated inhibitor I-1 suppresses the activity of protein phosphatase PP-1 (2,5,6).

In conclusion, the observed changes in phosphorylation of the total nuclear proteins in the chronic uncontrollable stress model are influenced by the concentration of second messengers in the cell nucleus (Ca^{2+} and cAMP). When the experimental impact is brief, the effect of the calcium ions predominates and consists of a strong activation of the Ca^{2+}-dependent protein kinases (protein kinase C and Ca^{2+}-calmodulin dependent). The processes of protein phosphorylation predominate over those of dephosphorylation. Prolongation of the experiment is probably paralleled by lowering of the concentration of the calcium ions, which determines the decrease in Ca^{2+}-dependent phosphorylation. The effect of cAMP was less manifest in the reported experimental model. A slight increase in phosphorylation and an inhibition of dephosphorylation occurred under the effect of the cyclic nucleotide.

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