Protein Kinase CK2 and Regulation of Ca^{2+}-ATPase Activity in Brain Neuron Chromatin in Rats during Aging

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 133, No. 6, pp. 653-655, June, 2002
Original article submitted April 18, 2002

Protein kinase CK2 from neuronal chromatin phosphorylates myosin-like proteins isolated from rat brain chromatin. The protein kinase CK2 activator 1-ethyl-4,5-di(N-methylcarbamoyl)imidazole *in vitro* stimulated phosphorylation of myosin-like proteins and increased myosin-type Ca^{2+}-ATPase activity in neuronal chromatin. After systemic administration this agent normalized Ca^{2+}-ATPase activity of brain chromatin in aged rats.

Key Words: *brain; aging; chromatin; protein kinase CK2; myosin-like proteins*

Aging is accompanied by impairment of regulatory systems in brain cells [10]. Properties of various contractile/cytoskeletal proteins in the nervous tissue, including β-tubulin, *tau*, MAP1B, actomyosin-like proteins, and dinein, change in patients with senile dementia and Alzheimer's disease [8,11]. These proteins determine cell cytoarchitectonics, structural and functional characteristics of membranes, and surface topography of neurons [8,11]. Contractile proteins myosin and actin are present in chromatin of various tissues [3,4]. Actomyosin-like proteins are probably involved in the organization of transcriptionally active chromatin [3]. Phosphorylation by various protein kinases plays an important role in the regulation of myosin activity [6]. In nonmuscle cells phosphorylation induces conformational changes in myosin and formation of the actomyosin complex, which is accompanied by the increase in ATP-hydrolyzing activity of myosin [6].

Our previous studies showed that protein kinase CK2 (PKCK2) activity and transcriptional activity of neuronal chromatin decrease in aged animals with pronounced cognitive disorders [5]. Learning in rats is accompanied by an increase in Ca^{2+}-ATPase activity, activation of PKCK2 in chromatin of cortical and hippocampal neurons, phosphorylation of HMG transcriptional factors, and intensification of transcription [1-3]. PKCK2 *in vitro* phosphorylates myosin heavy and light chains and troponin T [7,9]. It remains unclear whether PKCK2 is involved in the regulation of functional activity of contractile elements in chromatin. Here we studied the role of PKCK2 in the regulation of Ca^{2+}-ATPase activity in chromatin of brain neurons during aging.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats aging 7 (adult) and 24 months (aged). The rats were housed as follows: 180-200 g — 10 animals per cage, >400 g — 5 animals per cage. The animals were kept under controlled environmental conditions and had free access to food and water.

Selective PKCK2 modulators 1-ethyl-4,5-di(N-methylcarbamoyl)imidazole (EI) and 1-propyl-4,5-di(N-methylcarbamoyl)imidazole (PI) were injected intraperitoneally in a daily dose of 3 mg/kg for 7 days. Control animals received an equivalent volume of physiological saline. The rats were decapitated 1 h after the last injection.

Neuronal cells, nuclei, and chromatin were isolated from the cortex and hippocampus as described elsewhere [1,2].

Myosin-like proteins (MLP) were isolated from neuronal chromatin and purified [4].

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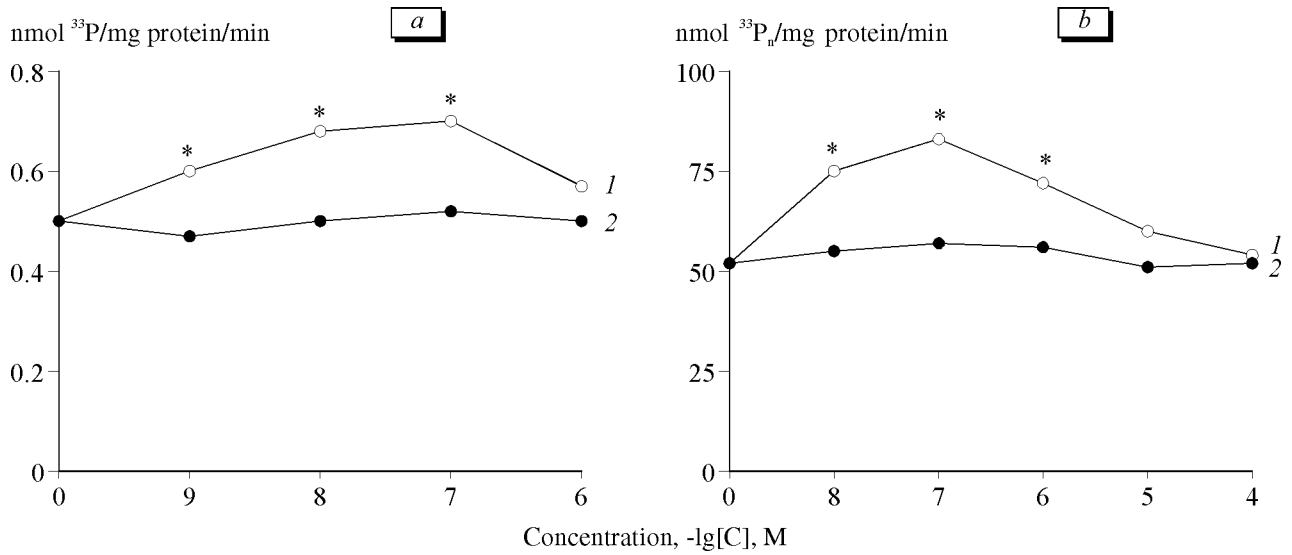

Fig. 1. Effects of protein kinase CK2 modulators on phosphorylation of myosin-like proteins (*a*) and myosin-type Ca^{2+}-ATPase activity in neuronal chromatin (*b*, *n*=5): 1-ethyl-4,5-di(N-methylcarbamoyl)imidazole (*1*) and 1-propyl-4,5-di(N-methylcarbamoyl)imidazole (*2*). *p<0.05 compared to the control.

Myosin-type Ca^{2+}-ATPase activity in chromatin was estimated by accumulation of pH in the medium in the presence of Ca^{2+}-ethyle glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid [3].

PKCK2 of neuronal chromatin was isolated from the cortex [1,2], purified, and incubated (2 mg/sample) with γ-^{33}P-ATP (molar activity 60-90 pBq/mol) at 30°C for 15 min in the presence of test compounds (10^{-8}-10^{-4} M). PKCK2 activity was estimated by ^{33}P incorporation [1,2]. MLP served as the substrate for phosphorylation (10 mg/sample).

Control and experimental groups included 10 or more animals. The results were analyzed by Student's *t* test.

RESULTS

PKCK2 of neuronal chromatin phosphorylates MLP isolated from rat brain chromatin (Fig. 1, *a*). The properties of these proteins were similar to those of myosin (*e.g.*, electrophoretic mobility and subunit composition). ATPase activity of proteins was typical of myosin, stimulated by Ca^{2+} in millimolar concentrations, and inhibited by Mg^{2+}. EI in concentrations of 10^{-9}-10^{-7} M accelerated phosphorylation of MLP with PKCK2 (Fig. 1, *a*). However, PI in the same concentration had no effect on the rate of MLP phosphorylation.

We revealed myosin-type Ca^{2+}-ATPase activity in neuronal chromatin [3]. EI in concentrations of 10^{-8}-10^{-6} M not only accelerated phosphorylation of MLP, but also increase Ca^{2+}-ATPase activity in neuronal chromatin from rat brain cortex and hippocampus (Fig. 1, *b*, hippocampal chromatin). PI in the same concentration did not modulate MLP phosphorylation and Ca^{2+}-ATPase activity. Thus, phosphorylation of MLP is accompanied by an increase in Ca^{2+}-ATPase activity in neuronal chromatin.

Age-related changes in chromatin can be associated with disturbances in phosphorylation of contractile elements. PKCK2 activity decreases in aged rats [5]. Our results show that PKCK2 phosphorylates MLP and, therefore, modulates Ca^{2+}-ATPase activity in chromatin. In 24-month-old rats Ca^{2+}-ATPase activity of chromatin decreased by 30 and 38% in cortical and hippocampal neurons, respectively, compared to that in adult animals (Fig. 2). The PKCK2 activator

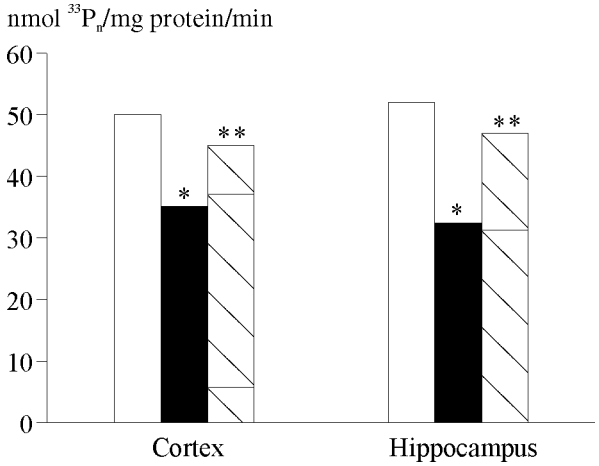

Fig. 2. Effects of the protein kinase CK2 activator on Ca^{2+}-ATPase activity in neuronal chromatin in aged rats (*n*=5). Light bars: 7-month-old rats. Dark bars: 24-month-old rats. Shaded bars: 24-month-old rats receiving 1-ethyl-4,5-di(N-methylcarbamoyl)imidazole. *p<0.05 compared to adult animals; **p<0.05 compared to aged animals.

increased Ca^{2+}-ATPase activity, which indicates that this enzyme plays an important role in the regulation of functional activity in contractile elements in chromatin. EI *in vitro* activates Ca^{2+}-ATPase, which suggests that this agent normalizes PKCK2-dependent regulatory mechanisms of transcription and attenuates the symptoms of age-related brain disorders in aged animals.

Experiments on aged animals showed that the specific PKCK2 activator enhances contractile activity of MLP in chromatin. After systemic administration EI increased Ca^{2+}-ATPase activity of chromatin in the cortex and hippocampus by 28 and 45%, respectively (Fig. 2). These changes were accompanied by an increase in transcriptional activity of neurons and attenuation of amnesia in aged rats [5]. Our results indicate that PKCK2 activators possess geroprotective activity.

This work was supported by the Russian Foundation for Basic Research (grant No. 02-04-49823).

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