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Effects of electroconvulsive shock on the phosphorylation of DARPP-32 in rat striatum

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ABSTRACT

Dopamine- and cAMP-regulated phosphoprotein with molecular weight 32 kDa (DARPP-32) is a key integrative molecule in the dopaminergic and glutamatergic signaling pathways in the striatum. Electroconvulsive shock (ECS), which induces massive neuronal depolarization, can activate various signaling pathways. In this study we investigated whether ECS could affect the phosphorylation status of DARPP-32. Male Sprague–Dawley rats underwent ECS and were sacrificed by decapitation at 0, 2, 10, 60, and 180 min after treatment. The phosphorylations of Thr34 and Thr75 residues of DARPP-32 and Ser159 residue of cyclin-dependent kinase 5 (CDK5) were investigated in the striatum. The activity of protein phosphatase 1 (PP1) and the binding between DARPP-32 and PP1 were also analyzed. Thr34 phosphorylation of DARPP-32 increased immediately after ECS and this state was maintained for more than 60 min. The activity of PP1 decreased and the binding between PP1 and DARPP-32 increased in accordance with this phosphorylation pattern. However, the phosphorylation at Thr75 showed no significant change except for an initial transient decrease. The phosphorylation of CDK5, which is responsible for Thr75 phosphorylation of DARPP-32, did not exhibit significant fluctuations. Our findings indicate that ECS increases Thr34 phosphorylation of DARPP-32, and thus inhibits the activity of PP1.

1. Introduction

Dopamine- and cAMP-regulated phosphoprotein with molecular weight 32-kDa (DARPP-32) is a key molecule in many neurotransmitter pathways throughout the brain, especially in the striatum (Greengard, 2001). Substantial evidence indicates that DARPP-32 is related to the pathophysiology of psychiatric disorders; DARPP-32 is known to mediate the brain's response to many drugs of abuse such as cocaine, opioids, and nicotine (Svenningsson et al., 2005), and modifications of DARPP-32 phosphorylation have been observed in cocaine-sensitized rats (Scheggi et al., 2007). The level of DARPP-32 was found to be decreased in the dorsolateral prefrontal cortex of patients with schizophrenia (Albert et al., 2002). Antipsychotic drugs specifically targeted DARPP-32-containing neurons in the nucleus accumbens shell (Ma et al., 2006). Finally, in DARPP-32 knockout mice, the antidepressant fluoxetine did not elicit a behavioral response (Svenningsson et al., 2002).

DARPP-32 is an inhibitor of protein phosphatase 1 (PP1) and protein kinase A (PKA). The function of DARPP-32 is regulated by threonine residue phosphorylation at two critical sites, Thr34 and Thr75. The phosphorylation at Thr34 is mediated by PKA and makes DARPP-32 a potent inhibitor of PP1 (Hemmings et al., 1984). Therefore, the activation of PKA inhibits the activity of PP1 via DARPP-32, amplifying the PKA-mediated phosphorylation signal. Dephosphorylation at Thr34 is achieved by protein phosphatase 2B (PP2B; Nishi et al., 2000), which is activated by calcium signaling. The phosphorylation of DARPP-32 at Thr75 results in inhibition of PKA (Bibb et al., 1999). This phosphorylation is mediated by cyclin-dependent kinase 5 (CDK5) (Bibb et al., 1999), which can be activated by calcium signaling via calpain and p35 (Nath et al., 2000). Thr75 is dephosphorylated by protein phosphatase 2A (PP2A; Nishi et al., 1999), and PP2A can be activated by either PKA or calcium signaling (Nishi et al., 2000, 2005). Thus, various phosphorylation–dephosphorylation networks are woven around DARPP-32.

DARPP-32 can be regulated by dopamine and glutamate in a complex manner. Dopamine can induce Thr34 phosphorylation of DARPP-32 by D_1 receptor-mediated activation of PKA, while D_2 receptor activation reduces Thr34 phosphorylation via the inhibition of PKA (Nishi et al., 2000). Glutamate can regulate Thr34 phosphorylation through various receptor types including the *N*-methyl-D-aspartate (NMDA) receptor, which activates PP2B and thus decreases Thr34 phosphorylation (Nishi et al., 2005). Similarly, Thr75 phosphorylation of DARPP-32, which is regulated by CDK5 and PP2A, can also be affected by both dopamine (D_1/D_2

Abbreviations: CDK5, cyclin-dependent kinase 5; DARPP-32, Dopamine- and cAMP-regulated phosphoprotein with molecular weight 32-kDa; ECS, Electroconvulsive shock; ECT, Electroconvulsive therapy; OA, Okadaic acid; pNPP, *p*-nitrophenyl phosphate; PKA, protein kinase A; PP1, protein phosphatase 1; PP2A, protein phosphatase 2A.

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receptors–PKA–PP2A sequence (Nishi et al., 2000)) and glutamate (NMDA receptor–calcium–CDK5/PP2A sequence; Nishi et al., 2005).

The striatum receives dopaminergic projections from the midbrain and glutamatergic projections from the cortical areas. In the striatum, hyperactivation of NMDA receptors has been shown to be related to neurological disorders such as Huntington's and Parkinson's diseases (DiFiglia, 1990; Greenamyre and O'Brien, 1991), and the pathophysiology of mood disorders and schizophrenia has been proposed to be related to the function of the striatum (Cummings, 1993; Yui et al., 1999). The biochemical changes elicited by psychotropic treatments in this region may contribute to the pathophysiology of various neuropsychiatric disorders.

Electroconvulsive therapy (ECT) has been widely used to treat psychiatric disorders including severe depression and schizophrenia. Convulsions induce massive and nonselective depolarization of neurons, and thus cause neurotransmitters to be discharged in a nonselective manner. Earlier human and animal studies suggested that ECT induces changes in many neurotransmitters including dopamine, serotonin, and glutamate (Fochtmann, 1994). Electroconvulsive shock (ECS), which is an animal model for ECT, activates various intracellular signal transduction pathways. The activity of protein kinases and phosphatases, the phosphorylation of transcription factors and the expression of immediate early genes are affected by ECS in various regions of the brain. For example, ECS increased the phosphorylation of cAMP response element binding protein (CREB) in the hippocampus (Jeon et al., 1997), suggesting the activation of PKA by ECS. Moreover, ECS also activated calcium signaling and PP2A activity (Jeon et al., 2001; Kang et al., 2005). Therefore, ECS probably affects the phosphorylation status of DARPP-32. However, the regulation of DARPP-32 phosphorylation is complex and it is difficult to predict the direction of change theoretically. Thus, we examined the pattern of phosphorylation of DARPP-32 in rat striatum after ECS.

2. Methods

2.1. Treatment of animals

Male Sprague–Dawley rats, weighing from 150 to 200 g, were treated in accordance with the 1996 NIH Guidelines for the Care and Use of Laboratory Animals (National Research Council, USA). The protocol was approved by the institutional review board. The rats were given ECS (130 V, 0.5 s, 60 Hz sine wave; model B24-III ECT apparatus; Medcraft Inc., Minami, FL, USA) via ear-clip electrodes and killed by decapitation at given times (0, 2, 10, 60, and 180 min after ECS). Time zero animals were decapitated immediately after the delivery of ECS. For other times, animals that showed typical tonic and clonic convulsions were selected for analysis. Sham animals were placed on the electrodes without real electric current and then decapitated. The striatum was immediately dissected and stored at −70 °C until analysis. Elapsed time from decapitation to freezing was less than 1 min.

2.2. Immunoblot analysis

The frozen tissues were homogenized in a glass-Teflon homogenizer in 10 volumes (v/w) of ice-cold homogenization buffer and centrifuged, as previously described (Roh et al., 2003). Homogenates containing 50 μg of protein were separated by 8% SDS-PAGE. Immunoblotting was performed using antibodies against phospho-DARPP-32 (Thr34 or Thr75; Cell Signaling Technology, Boston, MA, USA) and phospho-CDK5 (Ser159, Santa Cruz Biotechnology, Santa Cruz, CA, USA). Signals were detected using an enhanced chemiluminescence kit. The signals were quantitated with optical densitometry and normalized against corresponding signals from total forms (DARPP-32 antibody from Cell Signaling Technology; CDK5 antibody from Santa Cruz Biotechnology). It was recently reported that acute ECS did not alter the amount of DARPP-32 protein in the striatum (Rosa et al., 2007), and our experiment also confirmed this finding (data not shown).

2.3. Immunoprecipitation

For the immunoprecipation, tissue homogenates (1 ml, protein concentration of 2 mg/ml) were pre-cleared with protein-A sepharose and incubated overnight with an antibody against PP1 (2.5 μg, Santa Cruz Biotechnology). Immunoprecipitates were then harvested with protein-A sepharose, boiled with Laemmli's sample buffer and separated by 8% SDS-PAGE. Immunoblotting was performed with a DARPP-32 antibody, and then with a PP1 antibody. The ratio of DARPP-32 signal versus PP1 signal was then calculated.

2.4. Phosphatase assay

The phosphatase assay was performed using *p*-nitrophenyl phosphate (pNPP) as the substrate. Five microliters of tissue homogenate (2.5 mg/ml, homogenized without phosphatase inhibitors) was mixed with 80 μl of assay buffer [50 mM Tris (pH 7.0), 0.1 mM $CaCl_2$], 5 μl of 40 mM $NiCl_2$, and 5 μl of 5 mg/ml bovine serum albumin. The mixture was divided into three portions and preincubated for 30 min at 37 °C. Some samples were treated with 2.5 nM or 250 nM okadaic acid (OA) to inhibit the activity of phosphatases. Substrate [120 μl of 1.5 mg/ml pNPP in 50 mM Tris (pH 7.0)] was then added and incubated for 1 h. The optical density at 405 nM was measured using an ELISA reader. Incubation time was determined in a pilot experiment.

2.5. Statistical analysis

All experiments were repeated four times. Statistical analysis was performed by ANOVA with *post-hoc* Duncan test. Values of $P<0.05$ were considered significant.

3. Results

3.1. Thr34 phosphorylation of DARPP-32 after ECS

The phosphorylation at Thr34 of DARPP-32 changed significantly following ECS ($F=3.407$, $P=0.024$, $df=23$). It increased immediately (0 min) after ECS (Fig. 1a). At 2 min, phosphorylation was more than 200% from the sham level and this level was maintained until 60 min. At 180 min after ECS, the phosphorylation began to decline. A *post-hoc* test indicated that times 2 min to 180 min were differentiated from the sham. Time zero (0 min) was not differentiated from the sham, but also could not be differentiated from the other times.

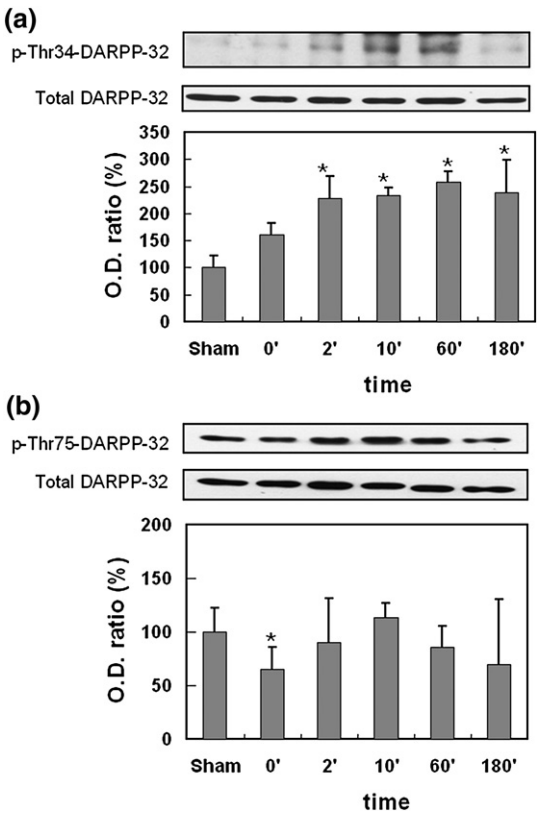

Fig. 1. Effects of electroconvulsive shock (ECS) on the phosphorylation of DARPP-32 in rat striatum. Rats were decapitated at the indicated times after ECS. Samples from the striatum were immunoblotted with phospho-Thr34-DARPP-32, phospho-Thr75-DARPP-32, and DARPP-32 antibodies. The optical densities of phospho-Thr34 and phospho-Thr75 signals were normalized against DARPP-32 signals from corresponding blots. Normalized values were compared with those of the sham. Four independent experiments were performed, and data are expressed as the mean ± S.E.M. of four sets of experiment. The asterisks (*) indicate statistically significant differences from the sham ($P<0.05$, ANOVA with *post-hoc* Duncan test). (a) phospho-Thr34-DARPP-32 (b) phospho-Thr75-DARPP-32.