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Research Report

DARPP-32 expression in rat brain after electroconvulsive stimulation

Daniela V.F. Rosa[a,b], *Renan P. Souza*[a,b], *Bruno R. Souza*[a,b], *Bernardo S. Motta*[a,b], *Fernando Caetano*[a,b], *Luciano K. Jornada*[c], *Gustavo Feier*[c], *Marcus V. Gomez*[b], *João Quevedo*[c], *Marco A. Romano-Silva*[a,b,*]

[a]*Grupo de Pesquisa em Neuropsiquiatria Clínica e Molecular, Universidade Federal de Minas Gerais, Av Antonio Carlos 6627, Belo Horizonte 31270-901 Minas Gerais, Brazil*

[b]*Departamento de Farmacologia, ICB, Universidade Federal de Minas Gerais, Av Antonio Carlos 6627, Belo Horizonte 31270-901 Minas Gerais, Brazil*

[c]*Laboratorio de Neurociências, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, 88806-000 Criciúma, SC, Brazil*

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ABSTRACT

Although electroconvulsive therapy (ECT) has been used as a treatment for mental disorder since 1930s, little progress has been made in the mechanisms underlying its therapeutic or adverse effects. The aim of this work was to analyze the expression of DARPP-32 (a protein with a central role in dopaminergic signaling) in striatum, cortex, hippocampus and cerebellum of Wistar rats subjected to acute or chronic electroconvulsive stimulation (ECS). Rats were submitted to a single stimulation (acute) or to a series of eight stimulations, applied one every 48 h (chronic). Animals were killed for collection of tissue samples at time zero, 0.5, 3, 12, 24 and 48 h after stimulation in the acute model and at the same time intervals after the last stimulation in the chronic model. Our results indicated that acute ECS produces smaller changes in the expression of DARPP-32 but, interestingly, chronic ECS increased transient expression of DARPP-32 in several time frames, in striatum and hippocampus, after the last stimulation. Results on the expression of proteins involved in signaling pathways are relevant for neuropsychiatric disorders and treatment, in particular ECT, and can contribute to shed light on the mechanisms related to therapeutic and adverse effects.

* *Corresponding author*. Departamento de Farmacologia – ICB, Universidade Federal de Minas Gerais, Av Antonio Carlos, 6627, 31270-901 Belo Horizonte-MG, Brazil. Fax: +5531 3499 2983.

E-mail address: romano-silva@ufmg.br (M.A. Romano-Silva).

Abbreviations: BDNF, brain derived neurotrophic factor; CRE, cyclic AMP responsive element; CREB, cyclic AMP response element binding protein; DA, dopamine; DARPP-32, dopamine and cAMP regulated phosphoprotein of Mr 32 kDa; D1R, dopamine receptor type 1; D2R, dopamine receptor type 2; ECS, electroconvulsive stimulation; ECT, electroconvulsive therapy; PI3K, phosphatidylinositide 3-kinase; PKA, cAMP-dependent protein kinase; PP1, protein phosphatase-1; Ser, serine; Thr, threonine; TrkB, tyrosine kinase receptor B; VEGF, vascular endothelial growth factor; 5-HTT, serotonin receptor; 5-HT_{1A}, serotonin receptor type 1_A; 5-HT_{2A}, serotonin receptor type 2_A

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1. Introduction

Electroconvulsive therapy (ECT) has been used as a treatment for mental disorders since 1930s. It is defined as a medical procedure in which a brief electrical stimulus is used to induce a cerebral seizure under controlled conditions (Rosen et al., 2003). Studies about ECT have progressed rapidly over the last 20 years, providing new insights into the mechanism of action, improving both its acute and long-term efficacy and decreasing cognitive problems associated with this treatment. Despite a large number of hypothesis on the mechanism of action of ECT have been proposed, it remains not well established. The main indications for ECT include depression, mania, catatonia and schizophrenia (Abrams, 1998). Particularly in the treatment of severe major depression, evidences for the effectiveness of ECT are clear and convincing (The UK ECT Review Group, 2003; Barichello et al., 2004).

Evidence for dopaminergic system dysregulation in depression is supported by a variety of reports, ranging from studies of dopamine (DA) and DA-metabolite levels, to neuroimaging, histopathological and neuroendocrine studies. Specifically, a number of reports suggest not only that depression may be linked to abnormally low dopamine levels, but also that the severity of depression is inversely correlated to central nervous system DA metabolite levels (Papakostas, 2006).

DARPP-32 (dopamine and cAMP regulated phosphoprotein of Mr 32 kDa) is a cytosolic protein that is selectively enriched in medium spiny neurons in the neostriatum. When DARPP-32 is phosphorylated by cAMP-dependent protein kinase (PKA) on Thr34, it is converted into a potent inhibitor of protein phosphatase-1 (PP-1). DARPP-32 Thr34 phosphorylation leads to an increase in the state of phosphorylation of downstream PP1 substrates, including several neurotransmitter receptors and voltage-gated ion channels (Greengard et al., 1999). In addition to Thr^{34}, DARPP-32 is phosphorylated at Thr^{75} by cyclin-dependent kinase 5 (Cdk-5). DARPP-32 phosphorylated at Thr^{75} inhibits PKA activity and thereby reduces the efficacy of DA signaling (Bibb et al., 1999). Svenningsson et al. (2006) showed that acute administration of fluoxetine in mice produced changes in phosphorylation of DARPP-32 in prefrontal cortex, hippocampus and striatum, while chronic treatment, in addition to phosphorylation changes, increased levels of DARPP-32 mRNA and protein. It has also been recently shown that the expression of DARPP-32 was downregulated in post-mortem brains of patients with schizophrenia (Albert et al., 2002).

Electroconvulsive stimulation (ECS) was shown to have many effects in experimental animals and those findings contributed toward a better understanding of the therapeutic and side effects of ECT (Newman et al., 1998). To our knowledge, no studies have examined the effect of acute or chronic ECS on DARPP-32 expression. To demonstrate changes in the protein expression profile after acute or chronic ECS, we analyzed DARPP-32 levels in the striatum, cortex, hippocampus and cerebellum of Wistar rats. Our results showed that acute ECS induced small changes in the expression of DARPP-32 but, interestingly, chronic ECS induced a sustained increase of the expression of DARPP-32 for several time frames after the last stimulation.

2. Results

2.1. *Effect of ECS on DARPP-32 expression in striatum*

DARPP-32 expression in the striatum of rats submitted to acute and chronic ECS was examined. Acute ECS did not show changes in expression levels of DARPP-32 during the time analyzed (Fig. 1A). DARPP-32 expression increased after chronic ECS at 3, 12 and 24 h when compared to sham and time zero groups ($p<0.05$). After 48 h, DARPP-32 expression returned to basal (Fig. 1B).

2.2. *Effect of ECS on DARPP-32 expression in cortex*

Twenty-four hours after acute ECS, DARPP-32 expression was increased compared to the sham group ($p<0.05$) (Fig. 2A). No difference was observed in DARPP-32 levels after chronic ECS (Fig. 2B).

2.3. *Effect of ECS on DARPP-32 expression in hippocampus*

DARPP-32 expression was not altered in the hippocampus after acute stimulation (Fig. 3A). The greatest increase of DARPP-32 levels (1888±180%), after chronic stimulation, was at time zero ($p<0.05$) when compared to sham group. When compared to time zero, differences were decreased in 30 min (338±18%), 3 h (860±112%), 12 h (676±101%), 24 h (559±2%) and 48 h (1339±29%) (Fig. 3B).

2.4. *Effect of ECS on DARPP-32 expression in cerebellum*

Acute or chronic ECS did not show alteration in DARPP-32 expression in cerebellum (Figs. 4A and B).

3. Discussion

ECS affects several brain regions, particularly hippocampus, frontal cortex, neostriatum, entorhinal cortex, temporal-parietal cortex and several monoaminergic nuclei that project into these areas (Fochtmann, 1994). It is known that sine wave ECT can lead to memory deficits and attention/executive functions deterioration (Fujita et al., 2006). DARPP-32 seems to be related to these processes (Heyser et al., 2000).

Acute ECS increased DARPP-32 levels in the cerebral cortex 24 h after stimulation. In all other regions examined (striatum, hippocampus and cerebellum), no differences were observed. Chronic ECS induced more significant changes in DARPP-32 expression in striatum and especially in the hippocampus. It has been proposed that the great benefits of ECT are derived from chronic treatment and repeated sessions in the clinical practice (Thienhaus et al., 1990).

Guitary and Nestler (1992) demonstrated that regulation of DARPP-32 immunoreactivity was induced by chronic administration of lithium; however, it was not observed in several other examined brain regions. Moreover, chronic administration of the antidepressant imipramine or tranylcypromine produced a similar increase in DARPP-32 levels in frontal cortex. Increased levels of DARPP-32 could reflect a common