Neuroscience Letters 554 (2013) 115–120

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Nongenomic, glucocorticoid receptor-mediated regulation of serotonin transporter cell surface expression in embryonic stem cell derived serotonergic neurons☆

Thorsten Lau[a,*,1], Felix Heimann[a,1], Dusan Bartsch[c], Patrick Schloss[a], Tillmann Weber[b,c]

[a] Department of Psychiatry and Psychotherapy, Biochemical Laboratory, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, J5, 68159 Mannheim, Germany
[b] Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, J5, 68159 Mannheim, Germany
[c] Department of Molecular Biology, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, J5, 68159 Mannheim, Germany

HIGHLIGHTS

- Generation of functional serotonergic neurons from a newly established murine C57BL/6N stem cell line.
- Dexamethasone induces rapid GR-mediated increase of 5-HTT cell surface expression.
- Increase in 5-HTT cell surface expression is independent of transcriptional regulation.

ARTICLE INFO

Article history:
Received 5 April 2013
Received in revised form 26 July 2013
Accepted 29 August 2013

Keywords:
Serotonergic neurons
Embryonic stem cells
Glucocorticoid receptor
Nongenomic regulation
Serotonin transporter

ABSTRACT

Depressive disorders have been linked to the combined dysregulation of the hypothalamus–pituitary–adrenal (HPA)-axis and the serotonergic system. The HPA-axis and serotonergic (5-HT) neurons exert reciprocal regulatory actions. It has been reported that glucocorticoid-glucocorticoid receptor (GR) signaling influences serotonin transporter (5-HTT) transcription but data also points to the fact that 5-HTT expression is regulated nongenomically *via* redistribution of 5-HTT from the cell surface into intracellular compartments. In order to analyze the acute effects of glucocorticoids on 5-HTT cell surface localization we differentiated serotonergic neurons from mouse embryonic stem (ES) cells derived from the C57BL/6N blastocysts. These postmitotic 5-HT neurons express all relevant serotonergic markers following the application of a growth factor-based differentiation protocol. Increasing concentrations of the GR agonist dexamethasone (Dex) resulted in enhanced, dose-dependent 5-HTT cell surface localization in the presence of the protein synthesis inhibitor cycloheximide already 1 h after incubation. Inhibition of GR function by the specific GR-antagonist mifepristone abolished the increase in 5-HTT cell surface localization. Hence, our data account for a nongenomic upregulation of 5-HTT cell surface expression by glucocorticoid–GR interaction which likely constitutes a rapid physiological response to increased levels of glucocorticoids as seen during stress. Taken together, we provide a cellular model to analyze and dissect glucocorticoid–5HTT interactions on a molecular level that corresponds to *in vivo* animal models using C57BL/6N mice.

1. Introduction

Dysregulation of the hypothalamus–pituitary–adrenal (HPA)-axis and the serotonergic (5-hydroxytryptamin, 5-HT) system have both been linked to the etiology and maintenance of anxiety and affective disorders [15,27]. One pivotal molecule in the 5-HT system is the serotonin transporter (5-HTT). 5-HTT is distributed between the cell surface and intracellular membranous structures and ongoing trafficking between these two compartments has been reported [5]. Only 5-HTT located in the cell membrane terminates serotonergic neurotransmission by the re-uptake of 5-HT from the extracellular space.

* Corresponding author. Tel.: +49 0621 1703; fax: +49 0621 17036205.
E-mail addresses: thorsten.lau@zi-mannheim.de (T. Lau), fheimann@gmx.net (F. Heimann), dusan.bartsch@zi-mannheim.de (D. Bartsch), patrick.schloss@zi-mannheim.de (P. Schloss), tillmann.weber@zi-mannheim.de (T. Weber).

[1] These authors contributed equally to this work.

0304-3940/$ – see front matter © 2013 Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.neulet.2013.08.070

In depressed patients, 5-HTT availability was found to be altered in imaging and postmortem studies [35,38]. Decreased 5-HTT availability correlated with an increased cortisol response after the combined dexamethasone suppression/CRH challenge (Dex/CRH) test [39].

5-HTT is selectively inhibited by selective serotonin re-uptake inhibitors (SSRIs) which are effective antidepressant and anxiolytic drugs. Chronically administered, they decrease 5-HT clearance by reducing 5-HTT cell surface expression [4,22] and normalize HPA-axis dysregulation in depressed patients [1,10,37].

In humans, a polymorphism (5-HTTLPR) in the promoter region of the 5-HTT gene (SLC6A4) has been shown to affect transcriptional regulation [31]. Stressful early-life events together with reduced 5-HTT expression in s/s-allele carriers have been associated with higher rates of depression [7] and higher cortisol levels upon stress exposure [18]. Also, s/s-allele carriers with unipolar depression showed a higher HPA-system activity in the Dex/CRH-test [48]. Hence, differential regulation of 5-HTT expression potentially involving the HPA-axis appears to be important for mood and anxiety disorders.

In order to investigate the molecular mechanisms of glucocorticoid-mediated regulation of 5-HTT expression in serotonergic neurons, we treated serotonergic neurons with the potent GR agonist Dex and studied its impact on 5-HTT cell surface expression. Because serotonergic neurons cannot be cultivated *ex vivo*, we have differentiated 5-HT neurons from embryonic stem (ES) cells derived from mouse C57BL/6 N blastocysts. Serotonergic differentiation was performed according to a protocol previously described [30] and yielded functional 5-HT neurons expressing 5-HT neuron-specific proteins. Escalating concentrations of Dex incrementally increased 5-HTT cell surface expression which could be blocked by pretreatment with the GR antagonist Mifepristone (Mif) but not with the protein-synthesis inhibitor cycloheximide. Hence, using a novel *in vitro* system of ES cell-derived 5-HT neurons we show a fast GR-mediated, gene expression-independent mechanism that regulates 5-HTT cell surface expression.

2. Material and methods

2.1. Mouse ES cells derived from C57BL/6N blastocysts

Blastocyst extraction and expansion of the inner cell mass was performed as described by Bryja and colleagues [6]. Briefly, mated females were killed E3.5 and their uteri dissected. Blastocysts were flushed out of the uterine horn and transferred into mouse embryonic fibroblasts (MEF) and SR-knockout medium (SRM; see supplementary data for all culture medium compositions) containing wells. After attachment of the blastocysts, the inner cell masses were allowed to expand for 4–5 days. Then, C57BL/6N-derived ES (B6-ES) cells were mechanically detached, dissociated with trypsine and plated in wells containing fresh MEF and 20% (v/v) FBS. The FBS containing medium was replaced by SRM the following day. A week later, compact cell clusters of typical ES cell morphology were detected, expanded and finally selected for further stem cell culture. Established B6-ES cells were cultured on MEF.

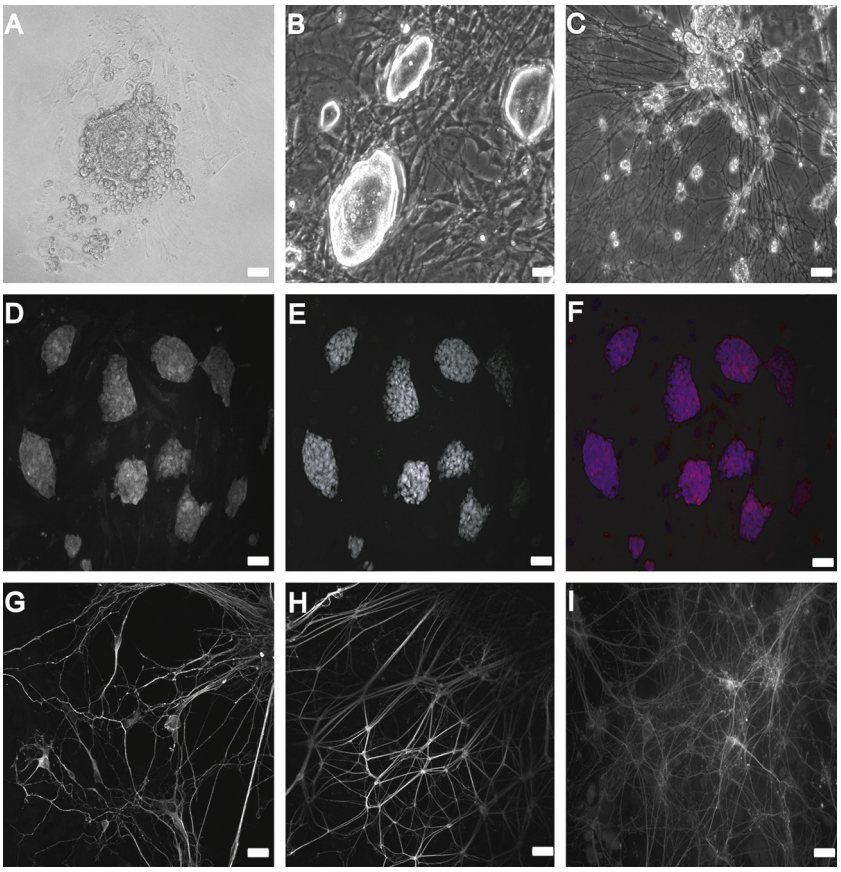

Fig. 1. Characterization and neuronal differentiation of B6-ES cells. (A) Hatching blastocyst on D5 after preparation from pregnant C57BL/6N mice. (B) B6-ES cell colonies growing on mouse fibroblast layer. (C) Representative network image of B6-ES cell-derived neurons 7 days after withdrawal of growth factors. (D) OCT4 immunostaining of B6-ES cell colonies grown on mouse fibroblasts. (E) Nuclear DAPI counterstaining. (F) Color merge of OCT4 immunostaining (red) and DAPI staining (blue). (G–I) Confocal z-stack projections of B6-ES cell-derived neurons immunostained for the neuronal markers MAP2a (G), TAU (H) and TuJ1 (I). Scale bars: 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)