Краткие сообщения

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Creation of the viral vectors for the inhibition of the serotonergic neurons using light sensitive proton pump

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Abstract. Depression is the most frequently diagnosed psychiatric disease in Western countries. Although a variety of pharmacological treatments for this disorder are available, a significant proportion of patients is treatment-resistant and/or suffer from side effects. There is a growing need for a complex understanding of the underlying pathogenic mechanisms in the neural circuits and effort for developing novel therapies for the depression. The role of serotonin in depression and antidepressant treatment remains unclear despite decades of research. New optogenetic tools for manipulation of neuronal activity have enabled the investigation of the contribution of distinct neural circuit elements to the pathogenesis of depression. In this study, we used the specific lentiviral vectors for the inhibition of serotonergic neurons. The proton pump archaerhodopsin-3 (eArchT3.0) has been expressed in the serotonergic neurons under the control of tryptophan hydroxylase 2 promotor. Green light stimulation of eArchT3.0 expressing neurons led to a reduced percentage of c-Fos expressing cells among those neurons, which indicates a decrease in their activity.

Keywords: serotonin, viral transfection, optogenetics, archaerhodopsin, raphe.

Introduction & aim

Serotonergic neurons are involved in the pathogenesis and the pharmacotherapy of major depression and anxiety disorders (Post et al. 2018). Yet, the precise mechanisms behind their involvement have not been determined. Moreover, available treatments (including selective serotonin reuptake inhibitors) seem to be effective in the treatment of depression, but more than two-thirds of depressed patients remain symptomatic after the initial intervention, and 20% of these fail to respond to any intervention (Gaynes 2009).

The recent development of optogenetic tools has made it possible to design new methods of treating mental disorders. Optogenetics has been widely used to control neuronal activity with high spatial and temporal resolution. There are many opsins that are used in optogenetics to excite or inhibit neuronal activity (Muir, Bagot 2019).

The outward proton pump enhanced ArchT3.0 (eArchT3.0) allows for efficient optogenetic silencing (Krol et al. 2019). This research was aimed at creating lentiviral vectors for the optogenetic inhibition of the serotonergic neurons using the proton pump archaerhodopsin-3.

Methods

To attenuate firing specifically in serotonergic neurons, vectors expressing the proton pump archaerhodopsin-3 under the control of TPH2 promoter were used. Viral vectors were constructed based on plasmids designed by the N. Nishitani group (Nishitani et al. 2019). Lentiviral particles (LVV) were obtained by transfecting HEK293 cells with the mixture of plasmids for the virus assembly (pPAX2 and pMD2.G), as well as a TPH2-eArchT3.0-eYFP-WPRE plasmid carrying the sequence of the archaerhodopsin-3 (eArchT3.0) and a yellow fluorescent protein (eYFP) or TPH2-Venus-WPRE plasmid that was used as a control. Wistar rats were stereotaxically injected with the virus in the dorsal raphe nucleus (DRN). One week after infection, animals were deeply anesthetized and green light (560 nm) was applied to the DRN for 3 min. Then animals were perfused, the brain was collected, frozen and sectioned. Expression of eArchT3.0-eYFP and c-Fos in the TPH-positive neurons was investigated immunohistochemically using confocal microscopy. The number of c-Fos, YFP, and TPH2 expressing neurons was determined. The expression of c-Fos was used as a marker of the neuron's activity.

Results

One week after the LVV injection, the specific expression of eArchT3.0-eYFP in serotonergic neurons was confirmed by immunohistochemistry. The majority of the TPH2 immunoreactive cells expressed eArchT3.0-eYFP that was detectable without immunohistochemical enhancement (fig. 1). Green light illumination for 3 min via optic fiber placed above the DRN decreased c-Fos expression in eYFP- or Venus-positive cells in TPH2-eArchT3.0 injected rats, compared with the TPH2-Venus injected rats (fig. 2). These observations demonstrated that continuous green light illumination for 3 min inhibited the activity of serotonergic neurons.

Conclusion

Our experiment demonstrated that the injection of TPH2-eArchT vectors in the DRN evokes the expression of the proton pump archaerhodopsin-3.

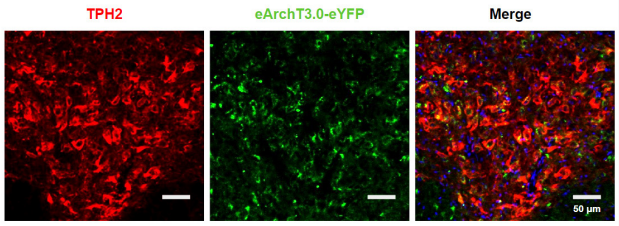

Fig. 1. The expression of eArchT3.0-eYFP in the tryptophan hydroxylase 2 (TPH2) immunopositive cells of the dorsal raphe nucleus, one week after the virus injection

We used c-Fos expression as a marker of neuronal activity to confirm whether green light illumination could inhibit the activity of the transfected serotonergic neurons. C-fos transcription can be induced by growth factors, cAMP signaling, and membrane depolarization (Flavell, Greenberg 2008). Synaptic activity regulates gene transcription by activating protein kinases associated with plasma membrane and then intracellular calcium-dependent signaling cascades that modify the function and/or expression of activity-dependent DNA-binding transcription factors such as c-Fos (Greer, Greenberg 2008). Green light illumination of eArchT3.0 induces outward current and suppressed action potential generation that leads to a decreased number of c-Fox expressing serotonergic neurons. Neuron membrane hyperpolarization state due to eArchT3.0 function could lead to a decrease in membrane protein kinase activity and, as a result, to a decrease in c-Fos expression.

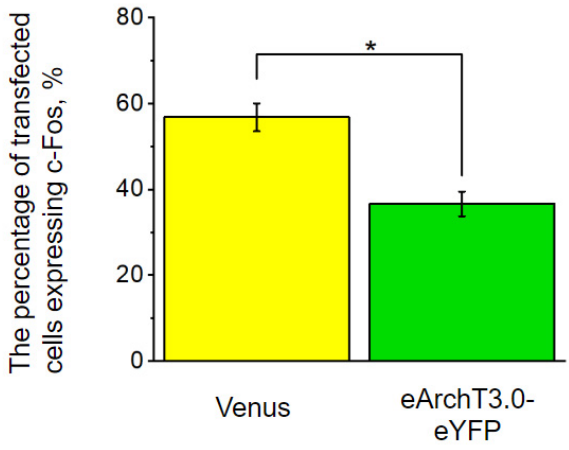

Fig. 2. Effects of optogenetic stimulation of the transfected serotonergic neurons on c-Fos expression. * $p < 0.001$ vs Venus

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