Molecular and Cellular Neuroscience 85 (2017) 148–161

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

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

Dual effect of serotonin on the dendritic growth of cultured hippocampal neurons: Involvement of 5-HT_{1A} and 5-HT_7 receptors

P.S. Rojas[a,1,2], F. Aguayo[a,2], D. Neira[a,2], M. Tejos[a], E. Aliaga[b], J.P. Muñoz[a], C.S. Parra[a], J.L. Fiedler[a,*]

[a] Laboratory of Neuroplasticity and Neurogenetics, Faculty of Chemical and Pharmaceutical Sciences, Department of Biochemistry and Molecular Biology, Universidad de Chile, Independencia, 8380492, Chile
[b] Department of Kinesiology, Faculty of Health Sciences, Universidad Católica del Maule, Talca, Chile

ARTICLE INFO

Keywords:
Serotonin
5-HT1AR
5-HT7R
Outgrowth
Dendrite
Hippocampal neurons
ERK1/2
AKT
GSK3β
LIMK
Cofilin

ABSTRACT

Serotonin acts through its receptors (5-HTRs) to shape brain networks during development and modulates essential functions in mature brain. The 5-HT_{1A}R is mainly located at soma of hippocampal neurons early during brain development and its expression gradually shifts to dendrites during postnatal development. The 5-HT_7R expressed early during hippocampus development, shows a progressive reduction in its expression postnatally. Considering these changes during development, we evaluated in cultured hippocampal neurons whether the 5-HT_{1A}R and 5-HT_7R change their expression, modulate dendritic growth, and activate signaling pathways such as ERK1/2, AKT/GSK3β and LIMK/cofilin, which may sustain dendrite outgrowth by controlling cytoskeleton dynamics.

We show that mRNA levels of both receptors increase between 2 and 7 DIV; however only protein levels of 5-HT_7R increase significantly at 7 DIV. The 5-HT_{1A}R is preferentially distributed in the soma, while 5-HT_7R displays a somato-dendritic localization at 7 DIV. Through stimulation with 5-HT at 7 DIV during 24 h and using specific antagonists, we determined that 5-HT_{1A}R decreases the number of primary and secondary dendrites and restricts the growth of primary dendrites. The activation of 5-HT_{1A}R and 5-HT_7R promotes the growth of short secondary dendrites and triggers ERK1/2 and AKT phosphorylation through MEK and PI3K activation respectively; without changes in the phosphorylation of LIMK and cofilin. We conclude that 5-HT_{1A}R restricts dendritogenesis and outgrowth of primary dendrites, but that both 5-HT_{1A}R and 5-HT_7R promote secondary dendrite outgrowth. These data support the role of 5-HT in neuronal outgrowth during development and provide insight into cellular basis of neurodevelopmental disorders.

1. Introduction

Serotonin (5-HT) is a chemical mediator that is expressed early during the development of the central nervous system (CNS) and it has been involved in important cognitive processes and human behavior (Lauder, 1993; Gaspar et al., 2003). Alterations in serotonergic neurotransmission have been linked to the appearance of several neuropathological disorders, including anxiety, depressive disorder, eating disorders, and others (Charney and Manji, 2004). It has been stressed that 5-HT plays a role during the early development of the CNS, which differs from its role in mature brain (Homberg et al., 2013). Several studies support a trophic role for 5-HT during CNS development by regulating cellular proliferation, migration, and neuronal differentiation (Lauder, 1993; Gaspar et al., 2003; Cote et al., 2007). Studies show that animals with a diet low in tryptophan (an amino acid precursor of 5-HT biosynthesis) show a decrease in dendritic arborization (Gonzalez-Burgos et al., 1996). Similarly, the abrogation of 5-HT synthesis using a knock-in mouse line in which the tryptophan hydroxylase 2 (Tph2) gene was replaced by the eGFP reporter gene showed severe abnormalities in the formation of serotonergic circuits (Migliarini et al., 2013). Furthermore, in vitro studies using hippocampal mouse neurons challenged with 5-HT have shown a reduction in the number of tertiary dendrites (Ferreira et al., 2010), indicating that this molecule could act through its receptors to sculpt neuronal morphology. Overall, these

* Corresponding author at: Laboratory of Neuroplasticity and Neurogenetics, Department of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, Sergio Livingstone 1007, Independencia, Santiago 8380492, Chile.
E-mail address: jfiedler@ciq.uchile.cl (J.L. Fiedler).
[1] Present address: Faculty of Medicine, School of Pharmacy, Universidad Andres Bello.
[2] Authors contributed equally to this study.

http://dx.doi.org/10.1016/j.mcn.2017.09.009
Received 22 May 2017; Received in revised form 4 September 2017; Accepted 29 September 2017
Available online 30 September 2017
1044-7431/

studies suggest that alterations of 5-HT levels during brain development affect proper neuronal wiring and therefore, may produce long-lasting modifications that affect brain functioning in adulthood.

5-HT acts through a large family of serotonin receptors, which are divided into seven subfamilies (5-HT_1-5-HT_7). Additionally, through alternative processing and editing of transcripts, these subfamilies can generate around 20 additional receptors, with different physiological functions (Mattson et al., 2004). With the exception of the 5-HT_3R, which is a ligand-gated ion channel, all serotonergic receptors are G-protein coupled, which positively or negatively regulate adenylate cyclase (AC). These receptors also promote PLC activation and therefore, generate IP_3 and diacylglycerol (DAG) (Mattson et al., 2004; Lesch and Waider, 2012). Immunohistochemical studies have shown early embryonic expression of these receptors, demonstrating a dynamic variation in their levels during pre- and post-natal development (Gaspar et al., 2003). To date, however, the specific contribution of each serotonergic receptor to the regulation of brain development and neuronal morphology has not been determined.

5-HT_{1A}R is one of the most studied serotoninergic receptors in relation to several pathologies (Dutton and Barnes, 2008). Its mRNA is detected in the foetal brain of rodents in the E12 stage at the raphe nucleus (Hillion et al., 1993) and in E16 at the hippocampus (Patel and Zhou, 2005), and it is also transiently expressed in hypoglossal motor neurons (Talley et al., 1997) and the cerebellum after birth (Miquel et al., 1994). Furthermore, depletion of 5-HT in the early postnatal period (P3) has no effect on the number and length of dendrites in hippocampal granule neurons, but it promotes a reduction of spine density (Yan et al., 1997a). This effect is reversed by buspirone, a 5-HT_{1A} agonist (Yan et al., 1997b), suggesting that 5-HT promotes the spinogenesis through the 5-HT_{1A}R. However, in vitro studies, using mouse neuroblastoma showed that the activation of 5-HT_{1A}R induces an increase in both the number and length of neurites (Fricker et al., 2005).

5-HT_7 R, the most recently identified member of the 5-HT receptor family (Volpicelli et al., 2014), is expressed in the hippocampus in early postnatal stages (P2-P6), and its expression decreases during later developmental stages (Kobe et al., 2012). In contrast to the 5-HT_{1A}R, the dynamic expression of the 5-HT_7R during postnatal development suggests a more restricted role at well-defined developmental stages. In mouse cultured hippocampal neurons, specific activation of 5-HT_7R promotes neurite elongation (Kvachnina et al., 2005). These evidences suggest that 5-HT_{1A}R and 5-HT_7R activation may impact the neuronal morphology. Our recently published study using rat hippocampal primary cultures demonstrated that both 5-HT_{1A}R and 5-HT_7R at 2 DIV promote the growth of secondary neurites, with no effect on neuritogenesis (Rojas et al., 2014). These results indicate that 5-HT_{1A}R and 5-HT_7R have redundant functions at early neuronal stages, in which neuronal polarity is established. Considering that the levels of these receptors change during development, we found it interesting to evaluate the role of these receptors in neuronal morphology at a more mature neuronal stage, i.e., when the arborization of dendrites is occurring.

Some studies have used overexpression of 5-HT_{1A}R and 5-HT_7R in non-neuronal cell lines to evaluate their coupling to several transduction pathways. For instance, the 5-HT_{1A}R has a wide spectrum of intracellular transducers, such as AC, potassium and calcium ion channels, and -similar to 5-HT_7R- is able to activate signaling pathways commonly associated with trophic responses, such as ERK and AKT kinases (Rojas and Fiedler, 2016; Wirth et al., 2016). Specifically, ERK and AKT signaling pathways have been related to cytoskeleton reorganization, possibly regulating dendritic arborization (Kim et al., 2004; Jaworski et al., 2005; Kumar et al., 2005). In addition, Rho GTPases such as Rac1 induce neurite growth and branching (Govek et al., 2005) through the phosphorylation and activation of LIM Kinase 1 (LIMK-1), which mediates actin cytoskeletal reorganization by phosphorylating the actin depolymerization factor cofilin (Endo et al., 2003). Furthermore, phosphorylated LIMK induces microtubule stability and actin polymerization in human endothelial cells (Gorovoy et al., 2005). Considering these antecedents, both 5-HT_{1A}R and 5-HT_7R may modulate dendrite outgrowth through similar or different signal transduction pathways depending on the cell phenotype of a particular developmental stage.

We evaluated variations of 5-HT_{1A}R and 5-HT_7R levels during the DIV of neuronal cultures, their location in neurons and the effect of the activation of these receptors on dendritic outgrowth and signaling pathways related to cytoskeleton dynamics. In contrast to the morphological changes observed in immature neurons, we determined that both receptors regulate dendritic morphology. 5-HT_{1A}R inhibits both the formation and growth of primary dendrites, but both 5-HT_{1A}R and 5-HT_7R promote the growth of secondary dendrites. Furthermore, we demonstrate a differential distribution of these receptors in the neuron; 5-HT_{1A}R is expressed in the neuronal soma and 5-HT_7R has a somatodendritic distribution. We also found that both receptors induce activation of ERK1/2 and AKT, with no changes in LIMK and cofilin phosphorylation. These modifications may explain, in part, the shared effect of both receptors on dendritic outgrowth.

2. Materials and methods

2.1. Ethics statement

Pregnant Sprague-Dawley rats were used in these experiments and were obtained from the Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile. The rats were handled according to guidelines outlined and approved by the Ethical Committee of the Faculty of Chemical and Pharmaceutical Sciences, Universidad de Chile, and the Science and Technology National Commission (CONICYT), and were in compliance with the National Institutes of Health Guide for the care and use of laboratory animals (NIH Publication, 8th Edition, 2011). Pregnant rats on gestational day 18 were euthanised after anaesthesia (ketamine 100 mg/kg and xylazine 10 mg/kg i.p.).

2.2. Hippocampal culture

Primary hippocampal neurons were cultured as previously described (Rojas et al., 2014). In brief, foetal rat hippocampal tissue (E18) were incubated in Hank's balanced solution with 0.05% trypsin (Biological Industries, Beit HaEmek, Israel). Digestion was stopped by serum addition and then centrifuged at 235*g* for 4 min. The pelleted cells were mechanically dissociated with a Pasteur pipette in DMEM supplemented with 10% foetal bovine serum. For immunocytochemistry studies, cells were plated at a density of 80,000 cells/well on 35-mm coverslips pre-coated with poly-D-lysine (0.1 mg/mL). After 2 h in the presence of 5% CO_2 at 37 °C, DMEM was replaced with an equal volume of neurobasal medium supplemented with 1% *v*/v B27, 2 mM Gluta-MAX, 1 mM sodium pyruvate, and 1000 IU/mL penicillin–streptomycin. After 2 days of culture, a mixture of mitosis inhibitor 5-fluoro-2-deoxyuridine/uridine (Sigma-Aldrich, St. Louis MO, USA) 4.4 μM was added for 24 h and was then replaced by fresh medium.

2.3. Evaluation of 5-HT_{1A}R and 5-HT_7R mRNA levels

Hippocampal primary cultures (10^6 cells) were lysed in TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Total RNA (2500 ng) was reverse transcribed into cDNA by using Superscript II (Invitrogen, Carlsbad, CA) and 250 ng of random primers (Invitrogen, Carlsbad, CA), following the manufacturer's protocol. Quantitative real-time polymerase chain reaction (RT-PCR) experiments were conducted with a specific set of primers to detect 5-HT_{1A}R and 5-HT_7R transcript levels, as we previously described (Rojas et al.,