PERSPECTIVE

Activation of β_2 adrenergic receptors promotes adult hippocampal neurogenesis

The subgranular zone (SGZ) of the hippocampal dentate gyrus is one of the few central nervous system regions where generation of adult born neurons occurs physiologically. This process, referred to as adult hippocampal neurogenesis (ahN), relies on the presence of neural progenitor cells (NPC) in the SGZ neurogenic niche. NPC, in response to appropriate cues and signals, give rise to neuroblasts migrating to the granular cell layer (GCL) where they mature into neurons functionally integrated into the hippocampal circuit (Toda et al., 2019). Although the functional role of adult born hippocampal neurons is not fully understood, a vast array of experimental evidence suggest that these cells are involved in crucial hippocampal-dependent functions like specific types of learning and memory and emotional responses (Toda et al., 2019). Intriguingly, deregulated ahN has been associated with several neurodegenerative and neuropsychiatric diseases. Major depression, in particular, is one of the disorders where dysregulated ahN has been more extensively studied (Boldrini et al., 2012; Bortolotto et al., 2014).

In recent years, our laboratory has been actively searching for novel modulators of ahN with potential therapeutic relevance. We could demonstrate that several drugs commonly utilized in the clinical settings are endowed with the ability of promoting or inhibiting ahN *in vitro* and/or *in vivo* (Bortolotto et al., 2014; Bortolotto and Grilli, 2017). Although at the preclinical level, our results suggested novel mechanisms of action and therapeutic indications for such drugs.

An intriguing and still undervalued aspect of ahN is its modulation by endogenous neurotransmitters. As an example, the SGZ niche receives a dense monoaminergic innervation (Toda et al., 2019). These findings are particularly interesting from a pharmacological perspective, since several clinically relevant drugs affect monoaminergic neurotransmission either directly or indirectly. Several lines of research provided evidence that classical antidepressants are positive modulators of ahN in human and rodent hippocampus. Moreover it has been proposed that the proneurogenic activity of antidepressants may, at least in part, contribute to the therapeutic effects of these drugs (Boldrini et al., 2012). While the role of serotonin receptor subtypes in ahN modulation has been extensively investigated both *in vitro* and *in vivo*, less is known about the role of adrenergic receptor (AR) in the regulation of ahN. This probably reflects the more extensive clinical use of Selective Serotonin Reuptake Inhibitors rather than antidepressants acting on noradrenergic transmission in depressed patients. Despite that, a particularly rich noradrenergic innervation of the SGZ originates in the locus coeruleus (LC), and there is also evidence that LC noradrenergic boutons make direct contact with NPC (Sara, 2009). Although β-ARs are of particular importance in several NA-mediated effects in the CNS, the specific contribution of distinct subtypes (β_1, β_2, β_3) remains to be fully understood. Based on these premises, we recently dissected the role of distinct β-AR subtypes, all functionally expressed by adult hippocampal NPC (ahNPC), in the modulation of ahN. Physiologically relevant noradrenaline concentrations significantly increased ahNPC proliferation rate *in vitro*, an effect which appeared to be mediated by β_1-AR engagement (Bortolotto et al., 2019). Conversely, β_2-AR subtype activation mediated noradrenaline effects on neuronal differentiation of adult hNPC. The contribution of β_2-AR to *in vitro* neurogenesis was confirmed by the fact that noradrenaline and β_2-AR agonists like salmeterol and formoterol were ineffective on neuronal differentiation in hNPC primary cultures isolated from β_2-AR$^{-/-}$ mice. Of note, β_3-AR stimulation could promote neurogenesis in β_2-AR$^{-/-}$ NPC cultures, confirming also this receptor subtype as positive modulator of ahN (Bortolotto et al., 2019). These *in vitro* observations prompted us to use β_2-AR agonists, molecules employed in the clinical setting as antiasthmatic drugs, as pharmacological tools in an *in vivo* study aimed at evaluating their activity as neurogenesis enhancers. For such purpose we selected salmeterol, a long-acting β_2-AR which crosses the blood-brain barrier. The drug, chronically (21 days) administered at a clinically relevant dose, significantly promoted neurogenesis in the adult murine hippocampus, as revealed by a remarkable increase in the number of BrdU$^+$/NeuN$^+$ newly generated neurons (Bortolotto et al., 2019). Of note, the number of total hippocampal BrdU$^+$ cells was not different between vehicle- and salmeterol-treated mice, suggesting that β_2-AR agonist administration had little/no effect on cell survival and/or proliferation in the adult neurogenic niche. This finding is in line with the *in vitro* observation that β_1-AR, rather than the β_2-AR subtype, is involved in the modulation of NPC proliferation. The proneurogenic effect of chronic salmeterol administration also correlated with a significant increase in the number of doublecortin (DCX) immunolabelled cells in the GCL of salmeterol-treated compared to vehicle-treated animals (Bortolotto et al., 2019). DCX is a microtubule associated protein expressed by immature neurons in the adult dentate gyrus, and it is commonly utilized as a marker of adult-born neuroblasts (Dellarole and Grilli, 2008). In the GCL, the radially oriented DCX$^+$ cell subpopulation is considered to represent a more advanced stage of maturation and migration compared to the cells which are tangentially oriented. Interestingly, in parallel with the increased number of DCX$^+$ cells, in salmeterol-treated mice we also observed a significant increase in the percentage of radially–compared to tangentially-oriented neuroblasts. The DCX marker is widely used for morphometric analysis of the dendritic arborizations of adult-born hippocampal neuroblasts, whose complexity is also a maturation index. Our analysis revealed a positive effect of chronic salmeterol administration on DCX$^+$ dendritic complexity and length, compared to vehicle-treated animals (Bortolotto et al., 2019). Altogether, our data suggested the idea that chronic salmeterol treatment increased hippocampal neuroplasticity by promoting both adult born neuroblast maturation and neurogenesis.

We believe that the findings of a novel pharmacological activity for salmeterol are worth of being further pursued in future studies. The noradrenergic neurotransmission plays key reg-

ulatory roles in a variety of physiological processes, including specific cognitive aspects which can be functionally correlated with ahN (Sara, 2009). Based on *in vitro* data, we suggest that adult hippocampal NPC and/or their DCX^+ progeny are cellular targets for the proneurogenic *in vivo* effects of salmeterol. Of course, at this stage of knowledge, we cannot exclude the possibility that other cell types may also contribute to mediate, either directly or indirectly, the proneurogenic effects of salmeterol. For example, β_2-AR stimulation in astrocytes, which express this receptor subtype, may result in modulation of expression and/or release of trophic factors and/or other soluble molecules which, in turn, may promote hippocampal neuroblasts maturation and neurogenesis (Cvijetic et al., 2017; Spampinato et al., 2019).

An unexpected finding of the chronic salmeterol study in adult mice was that the proneurogenic effects of salmeterol administration appeared to be restricted to ventral hippocampus (vHp). Region-specificity of salmeterol effects is a quite interesting observation since anatomical segregation along hippocampal dorso-ventral axis is an established concept (Grilli et al., 1988). Indeed, while dorsal hippocampus mainly receives inputs from cortical areas, vHp is more connected with subcortical structures, such as amygdala and the hypothalamic-pituitary-adrenal axis. Dorsal and ventral hippocampus are also segregated functionally: dorsal hippocampus has been correlated with cognitive functions such as spatial navigation, memory and learning, while the vHp with mood regulation, emotional behavior and buffering stress (Tanti and Belzung, 2013).

Chronic stress preferentially elicits deleterious effects on vHp neurogenesis and chronic antidepressant drugs have been shown to preferentially increase neurogenesis in rodent vHp (Tanti and Belzung, 2013). Moreover studies in depressed patients showed prominent effects of monoaminergic antidepressants in the anterior part of the hippocampus, the human correlate of rodent vHp (Boldrini et al., 2012). In such context, our demonstration that hippocampal neuroplasticity and neurogenesis induced by administration of chronic salmeterol occurs in the vHp of naïve adult mice, imposes that behavioural correlates of these effects should be further investigated. Specifically, the effects of salmeterol in animal models of stress-induced depressive-like behavior could be tested. Additionally, the possibility that salmeterol and/or β_2-AR agonists that pass the blood-brain barrier may enhance antidepressant effects exists and should be evaluated. In light of the fact that a third of patients suffering major depression do not satisfactorily respond or are resistant to antidepressant treatment, these observations certainly deserve further attention. Last but not least, ahN is also impaired in aging and in neurodegenerative diseases. As an example, Alzheimer's disease has been recently associated with a dramatic reduction in the number of adult born hippocampal neuroblasts, suggesting their degeneration in the disorder (Moreno-Jimenez et al., 2019). These observations suggest the need of evaluating the potential of β_2-AR agonists for preventing and/or attenuating the deterioration of hippocampal-associated functions, including cognitive impairment and mood disturbancies, in Alzheimer's disease and brain aging.

This work was partially supported by Progetti di Rilevante Interesse Nazionale (PRIN) 2017.

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***Received:** January 14, 2020*
***Peer review started:** February 10, 2020*
***Accepted:** March 35, 2020*
***Published online:** 20???*

***doi:** 10.4103/1673-5374.284991*

***Plagiarism check:** Checked twice by iThenticate.*
***Peer review:** Externally peer reviewed.*

***Open peer reviewers:** Sengal Bagci Taylan, Hakkari University, Turkey; Jie Liu, Jiangxi Province Center for Disease Control and Prevention, China.*
***Additional file:** Open peer review reports 1 and 2.*

References

Boldrini M, Hen R, Underwood MD, Rosoklija GB, Dwork AJ, Mann JJ, Arango V (2012) Hippocampal angiogenesis and progenitor cell proliferation are increased with antidepressant use in major depression. Biol Psychiatry 72:562-571.

Bortolotto V, Bondi H, Cuccurazzu B, Rinaldi M, Canonico PL, Grilli M (2019) Salmeterol, a β2 adrenergic agonist, promotes adult hippocampal neurogenesis in a region-specific manner. Front Pharmacol 10:1000.

Bortolotto V, Cuccurazzu B, Canonico PL, Grilli M (2014) NF-κB mediated regulation of adult hippocampal neurogenesis: relevance to mood disorders and antidepressant activity. Biomed Res Int 2014:612798.

Bortolotto V, Grilli M (2017) Opiates analgesics as negative modulators of adult hippocampal neurogenesis: potential implications in clinical practice. Front Pharmacol 8:254.

Cvijetic S, Bortolotto V, Manfredi M, Ranzato E, Marengo E, Salem R, Canonico PL, Grilli M (2017) Cell autonomous and noncell-autonomous role of NF-κB p50 in astrocyte-mediated fate specification of adult neural progenitor cells. Glia 65:169-181.

Dellarole A, Grilli M (2008) Adult dorsal root ganglia sensory neurons express the early neuronal fate marker doublecortin. J Comp Neurol 511:318-328.

Grilli M, Nisoli E, Memo M, Missale C, Spano P (1988) Pharmacological characterization of D1 and D2 dopamine receptors in rat limbocortical areas. II. Dorsal hippocampus. Neurosci Lett 87:253-258.

Moreno-Jimenez EP, Flor-Garcia M, Terreros-Roncal J, Rabano A, Cafini F, Pallas-Bazarra N, Avila J, Llorens-Martin M (2019) Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. Nat Med 25:554-560.

Sara SJ (2009) The locus coeruleus and noradrenergic modulation of cognition. Nat Rev Neurosci 10:211-223.

Spampinato SF, Bortolotto V, Canonico PL, Sortino MA, Grilli M (2019) Astrocyte-derived paracrine signals: relevance for neurogenic niche regulation and blood-brain barrier integrity. Front Pharmacol 10:1346.

Tanti A, Belzung C (2013) Neurogenesis along the septo-temporal axis of the hippocampus: Are depression and the action of antidepressants region-specific? Neuroscience 252:234-252.

Toda T, Parylak SL, Linker SB, Gage FH (2019) The role of adult hippocampal neurogenesis in brain health and disease. Mol Psychiatry 24:67-87.

P-Reviewers: Taylan SB, Liu J; C-Editors: Zhao M, Li JY; T-Editor: Jia Y