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The Endocannabinoid System and Neurogenesis in Health and Disease

ISMAEL GALVE-ROPERH, TANIA AGUADO, JAVIER PALAZUELOS, and MANUEL GUZMÁN
Department of Biochemistry and Molecular Biology I, School of Biology
Complutense University
Madrid, Spain

The endocannabinoid system exerts an important neuromodulatory function in different brain areas and is also known to be involved in the regulation of neural cell fate. Thus, CB_1 cannabinoid receptors are neuroprotective in different models of brain injury, and their expression is altered in various neurodegenerative diseases. Recent findings have demonstrated the presence of a functional endocannabinoid system in neural progenitor cells that participates in the regulation of cell proliferation and differentiation. In this Research Update, the authors address the experimental evidence regarding the regulatory role of cannabinoids in neurogenesis and analyze them in the context of those pathological disorders in which cannabinoid function and altered neuronal or glial generation is most relevant, for example, stroke and multiple sclerosis. NEUROSCIENTIST 13(2):109–114, 2007. DOI: 10.1177/1073858406296407

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The Endocannabinoid System

Endocannabinoids (eCBs) constitute a novel family of lipid ligands that act via specific G-protein–coupled receptors CB_1 and CB_2 (Piomelli 2003). The CB_1 receptor is widely expressed in the nervous system, with particular high levels in the neocortex, hippocampus, basal ganglia, cerebellum, and brainstem (Piomelli 2003). Functionally active CB_1 receptors are also expressed in peripheral nerve terminals and various extra-neural sites such as the testis, eye, vascular endothelium, and spleen. eCBs modulate neurotransmitter release and thus exert a wide array of actions including motor function, cognitive processes, emotion, sensorial perception, and endocrine functions and food intake (Mackie 2006). eCBs, namely, arachydonoylethanolamide (anandamide, AEA) and 2-arachidonoylglycerol (2AG), are produced on demand in a locally and temporally regulated manner by a calcium-dependent process (Fig. 1) (Piomelli 2003). Finally, eCBs are rapidly deactivated by reuptake mechanisms and degrading enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (Piomelli 2003; Mackie 2006). The CB_2 receptor displays a more limited pattern of expression than the CB_1 receptor, being found almost exclusively in cells (e.g., B- and T-lymphocytes, macrophages) and tissues (e.g., spleen, tonsils, lymph nodes) of the immune system (Klein 2005; Mackie 2006). Within the brain, the CB_2 receptor is only expressed in perivascular microglial cells, vascular endothelial cells, and certain neuron subpopulations.

Cannabinoid receptors initiate different signaling pathways including adenylyl cyclase inhibition and regulation of ionic channels: inhibition of voltage-dependent Ca^{2+} channels (N, P/Q type) and activation of inwardly rectifying K^+ channels (Mackie 2006). In addition, cannabinoids activate different protein kinase cascades (e.g., the phosphatidylinositol 3-kinase/Akt and the extracellular signal-regulated kinase [ERK]), modulate the generation of sphingolipid-derived signaling mediators and cell death pathways (e.g., caspase activation and the endoplasmic reticulum stress response) (Guzmán 2003). The eCB system can exert a pro-survival action of different neural cell types and thus is neuroprotective (Mechoulam and others 2002), but the opposite occurs with transformed cells that are driven to cells apoptosis by cannabinoid treatment and therefore exerts an antitumoral action against different types of cancer (Guzmán 2003). In summary, besides the important neuromodulatory role of the eCB system, cannabinoids are also involved in the control of neural cell fate, thereby modulating the balance between cell death and survival (Mechoulam and others 2002; Guzmán 2003).

Cannabinoid Regulation of Adult Neurogenesis

The initial finding of a cannabinoid regulatory action on adult neurogenesis via CB_1 receptors (Rueda and others

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Address correspondence to: Ismael Galve-Roperh, Department of Biochemistry and Molecular Biology I, School of Biology, José Antonio Novais sn, Complutense University, 28040 Madrid, Spain (e-mail: igr@quim.ucm.es).

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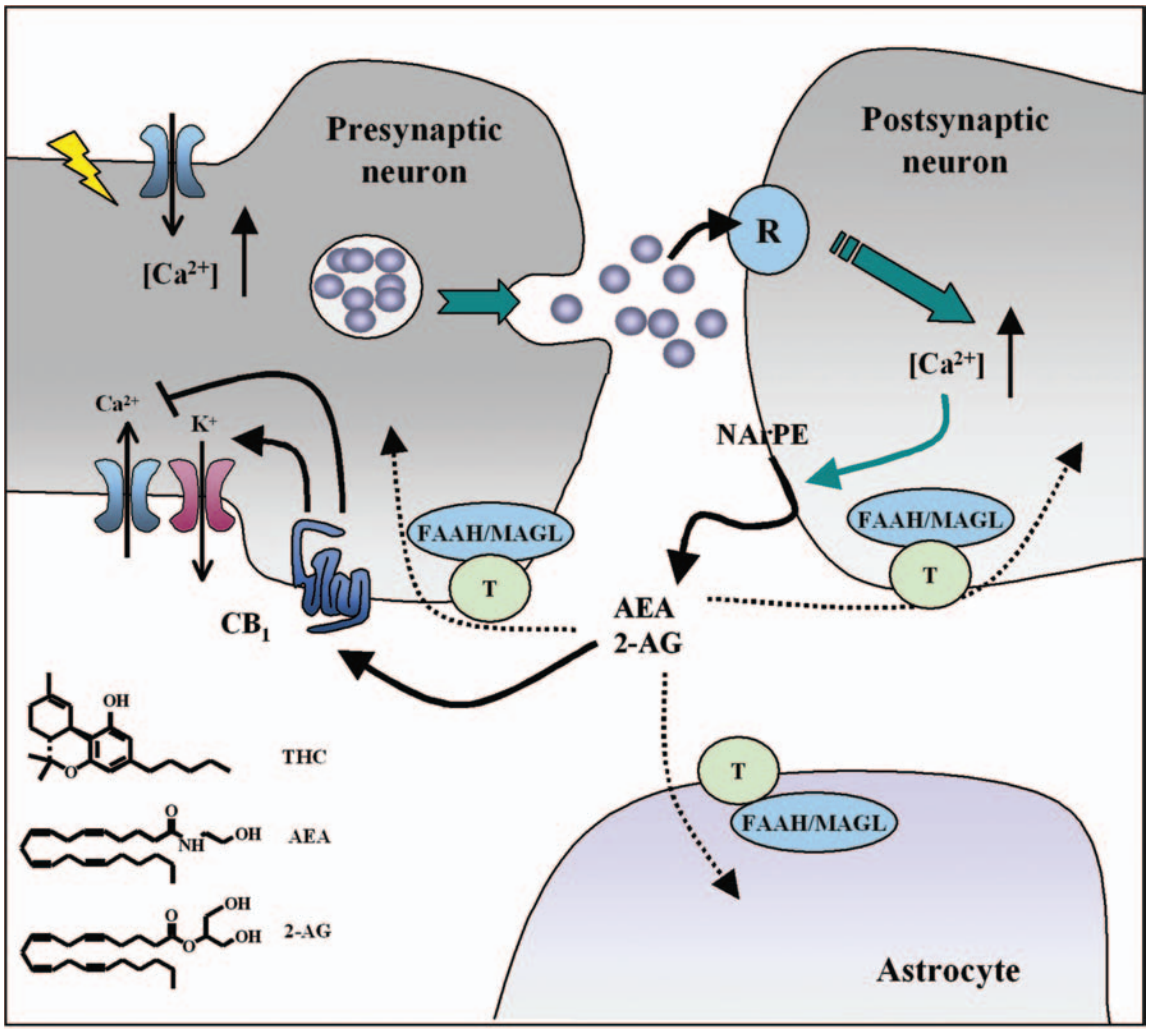

Fig. 1. Endocannabinoid production and turnover in the synaptic cleft. Presynaptic neuronal activation results in neurotransmitter release and induces an increase in intracellular calcium levels at the postsynaptic cell, thus activating AEA and 2AG production. Endocannabinoids act largely retrogradely and engage CB_1-receptor–mediated signaling pathways. Endocannabinoids have a fast turnover due to reuptake (T) and degradation enzymes (FAAH and MAGL). Plant-derived cannabinoid (e.g., Δ^9-THC) exerts its psychoactive effects by activating CB_1 receptors. AEA = anandamide; 2AG = 2-arachidonoylglycerol; CB = cannabinoid; FAAH = fatty acid amide hydrolase; MAGL = monoacylglycerol lipase; NArPE = N-arachidonoyl-phosphatidylethanolamine.

2002) was followed by the identification of the expression of the eCB system in neural progenitor cells (NPs) (Jin and others 2004; Aguado and others 2005). eCBs are produced by NPs, and knockout mouse studies have shown that they stimulate hippocampal- and subventricular zone-NP cell proliferation via CB_1 receptors (Jin and others 2004; Aguado and others 2005). Thus, CB_1-deficient mice show impaired NP proliferation, self-renewal, and neurosphere generation (Aguado and others 2005). In basal conditions, the higher content of AEA of FAAH knockouts induces astroglial cell generation (Aguado and others 2006), whereas pharmacological stimulation of CB_1 receptors may result in neurogenesis (Jiang and others 2005). These findings suggest that different types of agonists (endogenous or synthetic), pathophysiological situations (e.g., development or injury), and signal bioavailability (locally generated eCBs versus acute repeated injections of synthetic agonists) may modify the newly generated neural cell lineage by favoring a neuronal or a glial cell population. Thus, the anxiolytic and antidepressant effects induced by the administration of the synthetic cannabinoid HU-210 may be attributed as a functional consequence of the regulatory action of the eCB system on neurogenesis (Jiang and others 2005). Accordingly, CB_1-deficient mice have been shown to suffer from early age-related cognitive impairment (Bilkei-Gorzo and others 2005), one of the potential consequences of aging-associated decrease of neurogenesis (Lie and others 2004).

The role of the eCB system in the regulation of NP differentiation occurs in parallel with CB_1 receptor expression in vivo at different stages of brain development in embryonic (Fig. 2) (Aguado and others 2005; Berghuis and others 2005), postnatal (Aguado and others 2006),

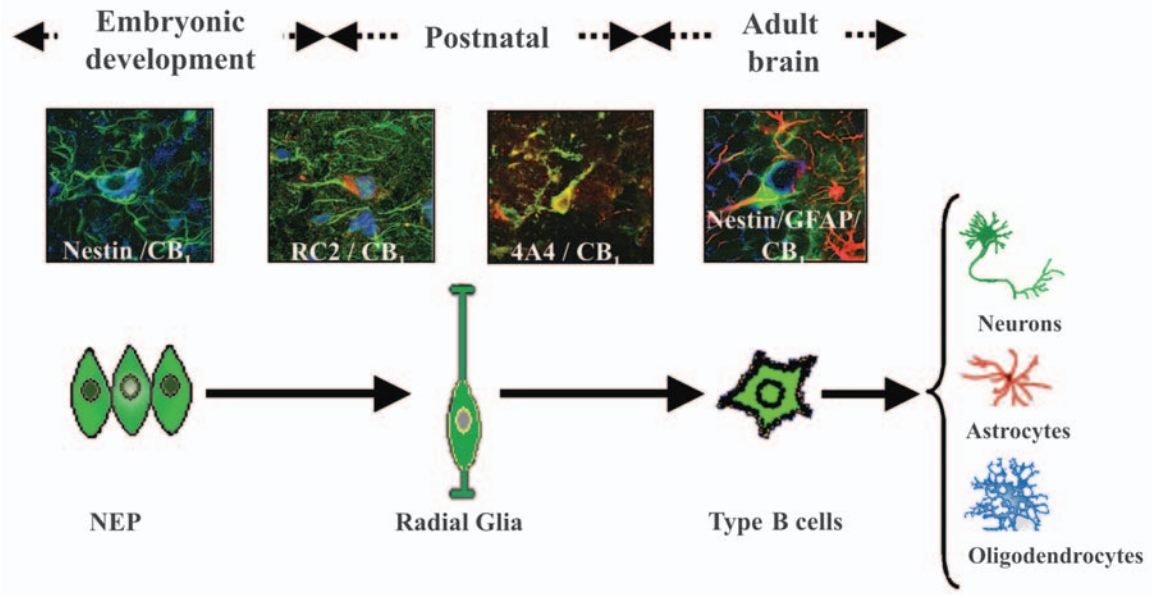

Fig. 2. Expression of CB_1 cannabinoid receptors by neural progenitors. CB_1 is expressed in vivo in cells that express the neuroepithelial progenitor (NEP) marker nestin or the radial glial marker RC2, in embryonic E17.5 and postnatal P2.5 mice, respectively. CB_1 is present in proliferating radial progenitors revealed by immunostaining phosphorylated vimentin 4A4. CB_1 receptors are also expressed by B-type cells (nestin$^+$ GFAP$^+$ cells) of the subgranular zone of the adult mouse hippocampus. CB = cannabinoid; GFAP = glial fibrillary acidic protein. From Aguado and others, *J Neurosci* 26(5):1551–61. Copyright 2006 by the Society for Neuroscience.

and adult hippocampal and subventricular NPs (Jin and others 2004; Aguado and others 2006). The expression of CB receptors in murine NPs has been extended to the hNSC1 embryonic human neural stem cell line (Rueda and others 2002; Palazuelos and others 2006) and an NP subpopulation of the adult human subependimal layer (Curtis and others 2006). These observations, together with the regulated pattern of expression of the eCB system, highlight the potential implications of the regulatory function of the eCB system in NPs during brain development (Fernández-Ruiz and others 2001).

Mechanism of Cannabinoid Action

The expression in NPs of the different eCB system elements, including receptors (CB_1, CB_2, TRPV1), endogenous ligands (AEA and 2AG), and the degrading enzyme FAAH, as well as the alterations of neurogenesis described in knockout mice (Jin and others 2004; Aguado and others 2005; Aguado and others 2006; Palazuelos and others 2006), supports a direct mechanism of action of eCB-initiated signal transduction pathways on NPs. Thus, cannabinoid regulation of NP cell fate may be attributed, at least in part, to their ability to regulate the ERK pathway (Rueda and others 2002; Palazuelos and others 2006). During cortical neurogenesis, sustained ERK signaling is required for neuronal generation and inhibition of gliogenesis. In this context, CB_1 activation regulates ERK activity dually in neuronal cells: CB_1-mediated inhibition of cortical NP and PC12 cell differentiation involves the attenuation of sustained ERK activity via Rap-1/B-Raf signaling inhibition (Rueda and others 2002), whereas NP proliferation and neuritogenesis of NPs and the neuroblastoma cell line Neuro-2A rely on ERK activation (Jordan and others 2005; Palazuelos and others 2006). The involvement of the down-regulation of nitric oxide production in CB_1-induced neurogenesis has also been proposed (Kin and others 2006).

The eCB system may exert its action by its cross-talking to growth factor signaling pathways that are essential for the expansion and functionality of NPs, as well as for neuronal survival and differentiation. In particular, basic fibroblast growth factor has been proposed to regulate neural cell growth by increasing 2AG generation (Williams and others 2003). Additionally, brain-derived neurotrophic factor production may be involved in cannabinoid-mediated neuroprotection after excitotoxicity (Marsicano and others 2003), and transactivation of TrkB receptors mediates CB_1 regulation of interneuron migration during embryonic development (Berghuis and others 2005). Moreover, the neuromodulatory function of the eCB system (Piomelli 2003) may contribute to the regulation of neurogenesis, as this process is controlled by neuronal activity (Lie and others 2004). The eCB system is therefore likely to be involved in the regulation of NPs via direct signaling, although as yet undescribed interactions with other pathways involved in neurogenesis cannot be ruled out.

Implications in Neurodegenerative Disorders

Great expectations have been generated by the recent demonstration that neural stem cell manipulation may be

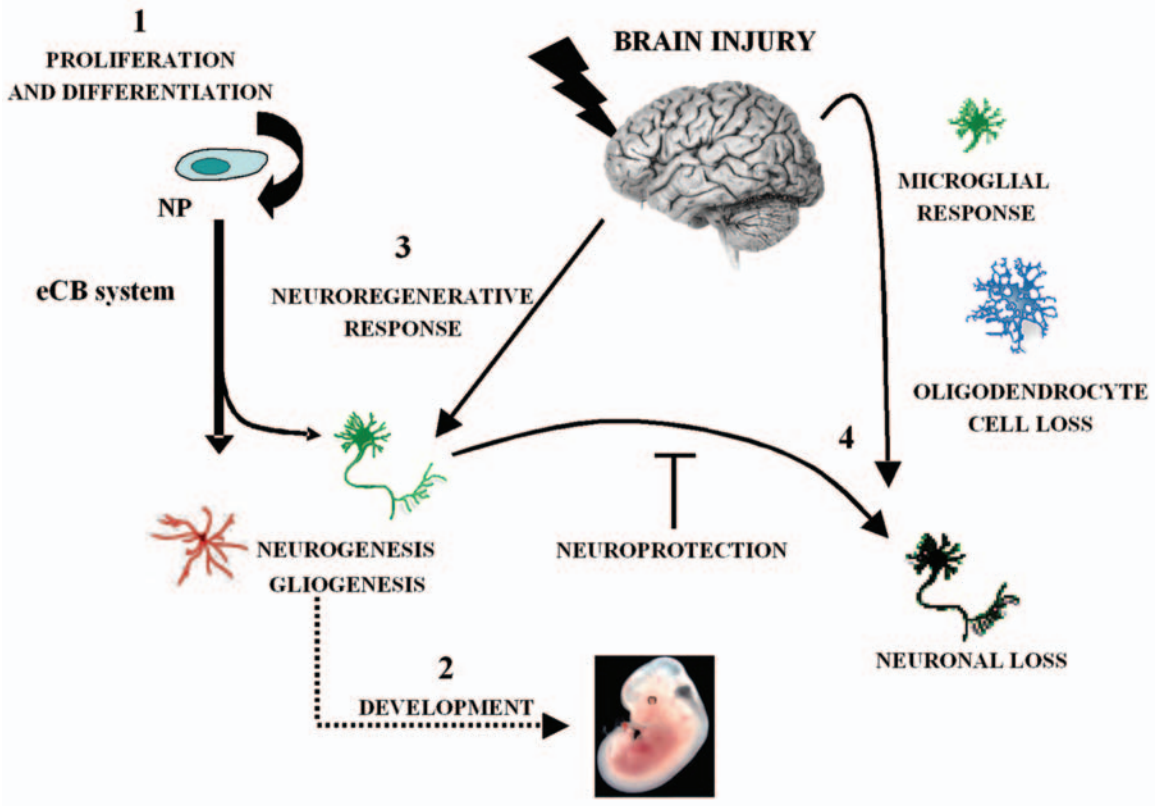

Fig. 3. Cannabinoids and neuroregeneration. The regulation of neural progenitor cell (NP) proliferation and differentiation (1) by the endocannabinoid (eCB) system may have implications during brain development, (2) injury-induced neurogenesis (3) (e.g., after stroke), or neuroprotection (4) against neuronal and oligodendrocyte cell loss (e.g., in demyelinating diseases).

useful in preclinical therapeutic strategies for the management of brain pathologies involving neural cell loss (Lie and others 2004). Thus, the identification of the endogenous signaling mechanisms responsible for the regulation of NPs constitutes one of the most active fields in stem-cell biology. Within the growing family of extracellular signaling systems involved in the regulation of NP cell fate (Lie and others 2004), the eCB system is an emerging candidate (Jin and others 2004; Aguado and others 2005; Jiang and others 2005; Aguado and others 2006). In addition to its neuroprotective role (Mechoulam and others 2002), eCB regulation of NP proliferation and differentiation (Fig. 3) constitutes a potential mechanism for some of the described therapeutic actions of cannabinoids in a variety of neurodegenerative disorders such as stroke (Nagayama and others 1999; Marsicano and others 2003), Alzheimer's disease (Ramirez and others 2005), and Huntington's disease (Glass and others 2004). Brain excitotoxicity engages on-demand activation of eCB signaling and results in neuroprotection of principal glutamatergic neurons (Marsicano and others 2003) and dopaminergic neurons (Melis and others 2006). Future research is, however, necessary to address whether under these pathophysiological circumstances the protective effects of the eCB system may involve the injury-induced regenerative response mediated by adult brain NPs (Lie and others 2004). In this context, excitotoxicity-induced brain damage induces the proliferation of hippocampal subgranular zone NPs, and this neurogenic response is lost in CB_1-deficient mice (Aguado, Guzmán, and Galve-Roperh, unpublished observations). Similarly, in Huntington's disease, in which the pathological grade may correlate inversely with neurogenesis, striatal CB_1 receptors are lost prior to the appearance of neurological deficits (Glass and others 2004), suggesting that altered cannabinergic activity may influence the development of Huntington's disease.

The involvement of the eCB system in NP regulation and its alterations in neurological disorders may provide in the future the basis for the design of novel avenues for the pharmacological manipulation of neurogenesis (Fig. 4). The development of such cannabinoid-based therapies would require strategies aimed at avoiding their potential psychoactive side effects (Mackie 2006). As these unwanted effects of cannabinoids are mediated largely or entirely by CB_1 receptors within the brain (Piomelli 2003), the design of selective cannabinoid ligands that target CB_2 receptors offers an attractive clinical possibility. In this context, CB_2 receptor activation has been shown to stimulate NP proliferation in vitro and in vivo (Palazuelos and others 2006). Alternatively, the use of inhibitors of eCB degradation or reuptake may enhance eCB signaling specifically in restricted brain areas upon

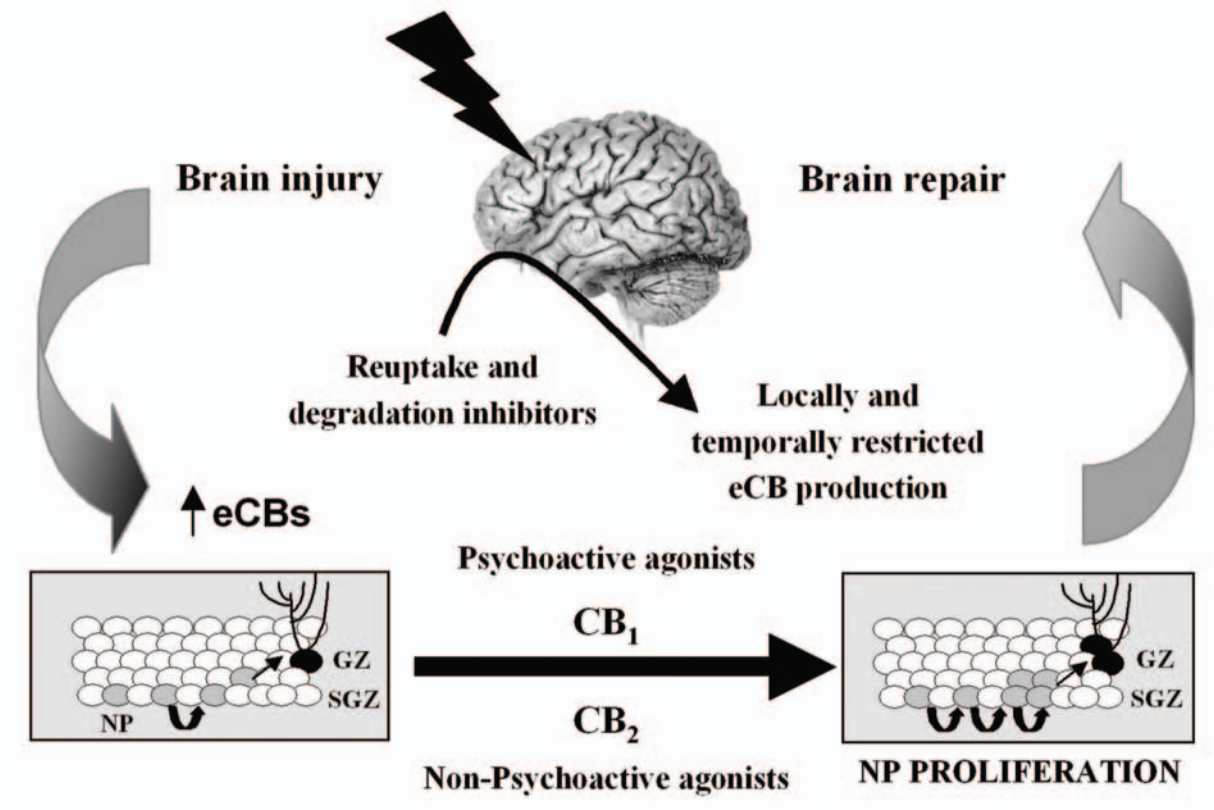

Fig. 4. Pharmacological strategies for cannabinoid manipulation of neurogenesis. CB_1 and CB_2 stimulation by synthetic cannabinoid agonists may increase NP proliferation and neurogenesis. Alternatively, the use of inhibitors of the deactivation elements of endocannabinoid signaling (inhibitors of endocannabinoid [eCB] reuptake and degradation) may result in on-demand eCB production in restricted brain areas. NP = neural progenitor cell; GZ = granular zone; SGZ = subgranular zone. From Palazelo and others, 2006. *FASEB* 20(13): 2405-7, with permission from the Federation of American Scientists for Experimental Biology.

on-demand eCB production, thus minimizing potential side effects.

Involvement in Demyelinating Diseases

Cannabinoids have been shown to be effective not only for palliating multiple sclerosis (MS) symptoms such as spasticity, tremor, neuropathic pain, and nocturia but also in animal models as neuroprotective agents (Pryce and Baker 2005). By attenuating neural cell loss, cannabinoids contribute to delayed progression of experimental autoimmune encephalitis (EAE) (Eljaschewitsch and others 2006). During EAE, altered eCB levels are observed in the brain and spinal cord (Pryce and Baker 2005) and regulation of the eCB tone with reuptake or degradation inhibitors (Ortega-Gutierrez and others 2005), as well as activation of CB_1 and CB_2 receptors, elicit improved motor symptoms (Arevalo-Martin and others 2003; Croxford and Miller 2003). Other beneficial actions of cannabinoids include oligodendrocyte-progenitor pro-survival action (Molina-Holgado and others 2002), inhibition of demyelination (Arevalo-Martin and others 2003), regulation of microglial activation (Eljaschewitsch and others 2006), and inhibition of T-cell infiltration (Arevalo-Martin and others 2003). In addition, cannabinoids exert an important regulatory action of the neuroimmunological status by favoring and inhibiting Th2 and Th1 immune responses, respectively (Klein 2005). Therefore, cannabinoid-mediated attenuation of neuroinflammation may cooperate in neuroprotection (Ramirez and others 2005; Eljaschewitsch and others 2006). The relevance of these findings is highlighted by the development of cannabinoid-derived medicines for the palliation of MS clinical symptoms (Pryce and Baker 2005). As a matter of fact, Sativex (a sublingual spray composed of Δ^9-tetrahydrocannabinol and cannabidiol) is administered in Canada for the management of MS-associated neuropathic pain (www.gwpharm.com). MS therapy must take into consideration that, besides inflammation, demyelination causes axonal loss and neurodegeneration, and therefore effective neuroprotection and remyelination are required for an efficient action in the secondary phase of the disease (Pryce and Baker 2005). Altogether, these findings suggest that in the future, cannabinoid-based

treatment of MS may go beyond the palliation of clinical symptoms and could also target the etiological mechanisms responsible for the disorder.

Concluding Remarks and Future Directions

Recent findings support the involvement of the eCB system in the regulation of NP cell fate and add to its previously described roles in the brain, including neuroprotection and the modulation of synaptic plasticity and neuronal excitability. Future research is required to identify the precise cell signaling mechanisms involved in eCB regulation of NPs, either as instructive signaling cues per se or by influencing other endogenous signals known to be involved in the neurogenic or gliogenic pathways. A crucial aspect of neuronal cell generation induced by cannabinoids, as yet undetermined, is the specific differentiation program engaged and thus the functional neurotransmitter phenotype of newly formed neurons. In addition, further research is required to elucidate the precise role of the eCB system in the regulation of neurogenesis during brain development and neurodegenerative disorders.

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