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Serotonin 1A receptor agonist increases species- and region-selective adult CNS proliferation, but not through CNTF

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ABSTRACT

Endogenous ciliary neurotrophic factor (CNTF)[1] regulates neurogenesis of the adult brain in the hippocampal subgranular zone (SGZ)[2] and the subventricular zone (SVZ)[3]. We have previously shown that the cAMP-inhibiting D2 dopamine receptor increases neurogenesis by inducing astroglial CNTF expression. Here, we investigated the potential role of CNTF in the proliferative response to pharmacological stimulation of the serotonin 1A (5-HT1A)[4] receptor, which also inhibits cAMP, in adult mice and rats. Like others, we show that systemic treatment with the active R-enantiomer of the 5-HT1A agonist 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT)[5] induces proliferation in the SGZ in rats using unbiased stereology of 5-Bromo-2′-deoxyuridine (BrdU)[6] positive nuclei. However, despite the bioactivity of R-8-OH-DPAT, as also shown by a decrease in hippocampal nNOS[7] mRNA levels, it did not increase CNTF mRNA as shown by highly specific quantitative RT-PCR (qPCR)[8]. Surprisingly, R-8-OH-DPAT did not cause an increase in SVZ proliferation in rats or in either the SVZ or SGZ of two different strains of mice, C57BL/6J, and 129SvEv, using acute or chronic treatments. There also were no changes in CNTF mRNA, and also not in mice treated with a widely used racemic mixture of 8-OH-DPAT, higher doses or after intracerebral injection, which reduced nNOS. In contrast to the others, we propose that the 5-HT1A receptor might be non-functional in mice with regards to regulating normal neurogenesis and has region-selective activities in rats. These species- and region-specific actions raise important questions about the role of the 5-HT1A receptor in human neurogenesis and its implications for the field of depression.

1. Introduction

Neurogenesis in the adult mammalian brain occurs predominantly in the SVZ of the anterior lateral ventricles and the SGZ of the hippocampal dentate gyrus (Hagg, 2009; Kriegstein and Alvarez-Buylla, 2009; Ming and Song, 2005; Soumier et al., 2010; Suh et al., 2009). SVZ neuroblasts migrate along the rostral migratory stream to the olfactory bulb and SGZ neuroblasts migrate into the neighboring granule cell layer where they become integrated neurons or die (Lois and Alvarez-Buylla, 1994; Luskin, 1993; Winner et al., 2002).

Many endogenous molecules regulate adult CNS neurogenesis (Bath and Lee, 2010; Hagg, 2005, 2009; Lie et al., 2004; Ming and Song, 2005; Suh et al., 2009). CNTF is responsible for ~30% of SVZ neurogenesis as shown in CNTF−/− mice and by antibody injections (Emsley and Hagg, 2003; Yang et al., 2008). We study CNTF because it is produced almost exclusively in the nervous system (Ip, 1998; Stockli et al., 1989) and would therefore be a good target for indirect stimulation of neurogenesis by systemic small molecule drugs. We found that nigrostriatal projections induce neurogenesis via D2 dopamine receptors, which is entirely mediated by astroglial CNTF (Baker et al., 2004; Yang et al., 2008). This is consistent with the fact that D2 receptors inhibit cAMP in astrocytes (Kalkman et al., 2003; Vallar and Meldolesi, 1989), whereas CNTF expression is inhibited by cAMP in astrocytes (Carroll et al., 1993; Rudge et al., 1994). CNTF

Abbreviations: CNTF, ciliary neurotrophic factor; SGZ, subgranular zone; SVZ, subventricular zone; 5-HT1A, serotonin 1A; 8-OH-DPAT, 8-Hydroxy-2-(di-n-propylamino)tetralin; BrdU, 5-Bromo-2′-deoxyuridine; nNOS, neuronal Nitric Oxide Synthase; qPCR, quantitative real-time reverse transcription-polymerase chain reaction; HF, hippocampal formation; CREB, cAMP response element binding protein; L-NAME, N5-[imino(nitroamino)methyl]-L-ornithine, methyl ester, monohydrochloride.

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is predominately produced by astrocytes (Dallner et al., 2002; Sendtner et al., 1994) which are spared in most neurodegenerative diseases. Therefore, they are ideal pharmacological targets to serve as CNTF "factories" to enhance endogenous proliferation. This would also circumvent the peripheral side effects and low bioavailability seen with systemic administration of CNTF (Thoenen and Sendtner, 2002).

We wanted to find additional cAMP-inhibiting drugs to increase CNTF, as they could be combined with low doses of D2 agonists to reduce systemic drug doses and, consequently, side effects. The 5-HT1A receptor is expressed on a subset of astroglia throughout the brain, in addition to neurons in the raphe nuclei (Whitaker-Azmitia et al., 1993). Their activation decreases cAMP levels (Azmitia, 2001; Kalkman et al., 2003; Mendez et al., 1999; Vanhoose et al., 2004). Colocalization of 5-HT1A receptor and GFAP is among the highest in the polymorphic layer of the dentate gyrus (Whitaker-Azmitia et al., 1993). Interestingly, serotonergic and dopaminergic projections to the SVZ overlap where neurogenesis and high expression of CNTF are located (Hagg, 2005). Activation of 5-HT1A receptor increases neurogenesis in the SVZ and SGZ in rats (Banasr et al., 2004; Brezun and Daszuta, 1999; Huang and Herbert, 2005; Soumier et al., 2010). A landmark study (Santarelli et al., 2003) showed that hippocampal neurogenesis in 129SvEv mice and the effects of antidepressants are regulated through 5-HT1A receptors, using 5-HT1A receptor −/− mice and 8-OH-DPAT. Acute but not chronic 8-OH-DPAT treatment increases SGZ proliferation in C57BL/6J mice (Klempin et al., 2010). Here, we tested whether CNTF mediates the proliferative effects of 8-OH-DPAT in the SVZ and SGZ of adult C57BL/6J and 129SvEv mice, as compared to rats.

2. Material and methods

2.1. Animals

All animal procedures were performed according to University of Louisville Institutional Animal Care and Use Committee protocols and the National Institutes of Health guidelines. In addition, every effort was made to minimize animal suffering and reduce the number of animals used.

A total of 153 mice were used, i.e., 101 C57BL/6J adult male mice (8–12 weeks, 20–30 g, stock # 000664, Jackson Laboratory, Bar Harbor, ME, USA), 40 129SvEv adult male mice (model # 129SVE-M [129S6/SvEvTac], 11 weeks, 20–30 g, Taconic, Hudson, NY, USA), and 12 wild type background of our CNTF mice (essentially C57BL/6) were used. In addition, 10 adult male Sprague-Dawley rats (280–350 g, Harlan, Indianapolis, IN) were used. The average weight of the animals in each experimental group was the same. All invasive procedures in mice were performed under deep anesthesia obtained by an intraperitoneal injection of 0.4 mg/g body weight Avertin (2,2,2-tribromoethanol in 0.02 ml of 1.25% 2-methyl-2-butanol in saline, Sigma-Aldrich, St Louis, MO, USA). Rats were anesthetized with an intramuscular injection (2.5 ml/kg) of a mixture containing 5 ml of ketamine (500 mg/ml, Hospira, Lake Forest, IL), 0.5 ml of acepromazine (10 mg/ml, ButlerSchein, Dublin, OH), and 1.2 ml xylazine (20 mg/ml, Akorn, Decantur, IL) diluted in 13.3 ml of 0.9% saline.

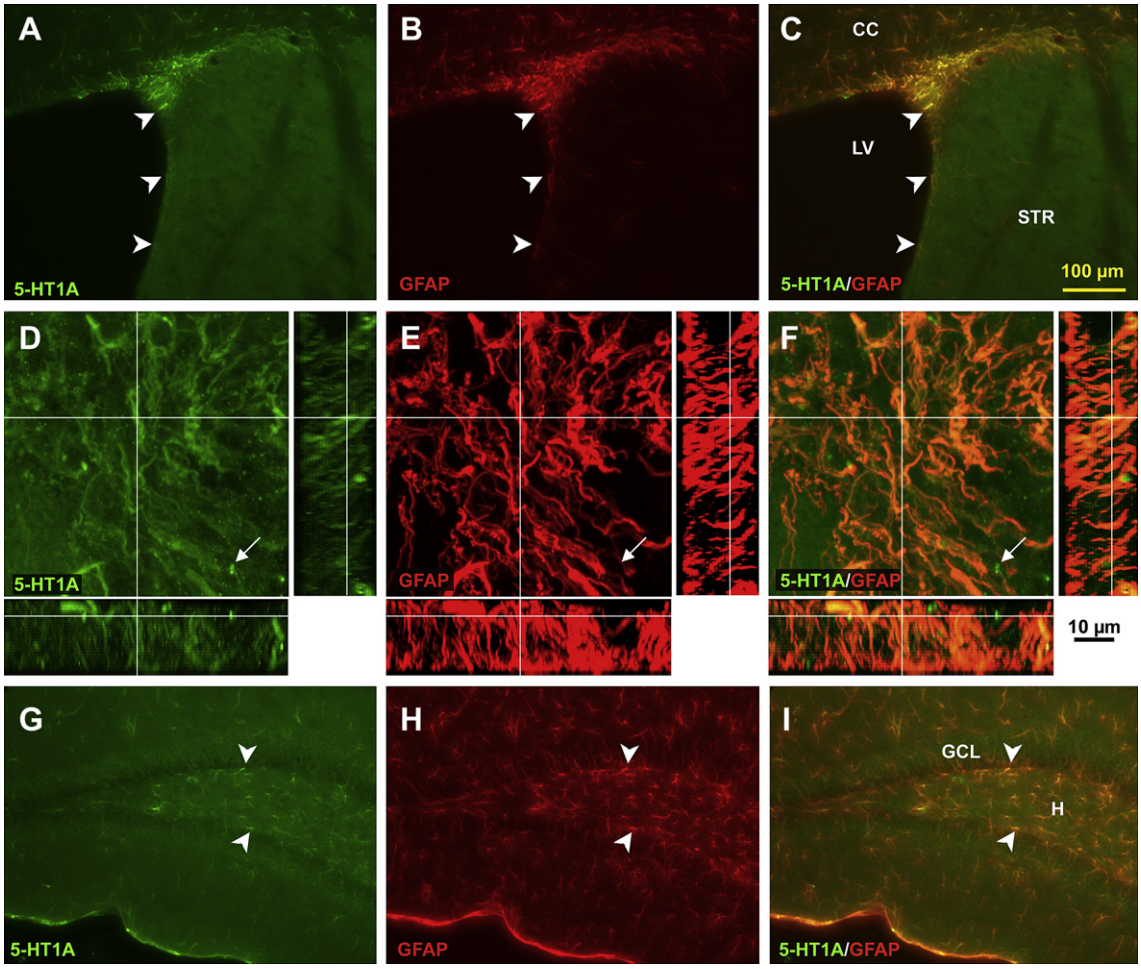

Fig. 1. 5-HT1A receptors are present in astrocytes in the SVZ and SGZ of adult C57BL/6J mice. 5-HT1A receptor (A) and GFAP (B) are co-localized (C) in the SVZ. Confocal microscopy shows that 5-HT1A receptor (D) and GFAP (E) are almost exclusively co-localized (F), as indicated in the z-stack, except for a few putative axon terminals, presumably presynaptic receptors of serotonergic projections (arrows). In the dentate gyrus, 5-HT1A receptor (G) and GFAP (H) also shows co-localization (I), including in the SGZ. Scale bar 100 µm (A–C, H–I), 10 µM (D–F). CC = corpus callosum, GCL = granule cell layer, H = hilus, LV = lateral ventricle, STR = striatum.