Available online at www.sciencedirect.com

SCIENCE DIRECT

Experimental Neurology 194 (2005) 444 – 456

Experimental Neurology

www.elsevier.com/locate/yexnr

Activation of noradrenergic transmission by α_2-adrenoceptor antagonists counteracts deafferentation-induced neuronal death and cell proliferation in the adult mouse olfactory bulb

Alexandra Veyrac[a,b], Anne Didier[a,b], Francis Colpaert[c], François Jourdan[a,b,*], Marc Marien[c]

[a]*Laboratoire Neurosciences et Systèmes Sensoriels, CNRS-UMR 5020, Université Claude Bernard-Lyon 1, 50 Avenue Tony Garnier, F-69366 Lyon, France*

[b]*Institut Fédératif des Neurosciences de Lyon IFR19, 59 bd Pinel, 69394-Lyon, France*

[c]*Centre de Recherche Pierre Fabre, 17 Avenue Jean Moulin, 81106 Castres, France*

Received 1 February 2005; revised 2 March 2005; accepted 7 March 2005
Available online 22 April 2005

Abstract

The olfactory bulb is the target of neural progenitor cells that are generated in the subventricular zone of the lateral ventricle in the adult brain. This permanent neurogenesis is likely influenced by olfactory input to the bulb since previous studies have shown that cell proliferation and/or apoptotic death are stimulated by naris closure or surgical transection of the olfactory nerve. Since the olfactory bulb is densely innervated by noradrenergic afferents originating in the locus coeruleus, we have studied the impact of pharmacologically activating this noradrenergic system on cell death and proliferation following unilateral olfactory axotomy in the adult mouse olfactory bulb. We found that noradrenaline release in the olfactory bulb was significantly increased by intraperitoneal injections of the selective α_2-adrenoceptor antagonists, dexefaroxan (0.63 mg/kg) and 5-fluoro-methoxyidazoxan (F 14413; 0.16 mg/kg). A chronic treatment with either compound for 7 days following olfactory axotomy significantly reduced neuronal death, glial activation and cell proliferation in the deafferented olfactory bulb. These data (1) confirm that α_2-adrenoceptor antagonists, presumably by facilitating central noradrenergic transmission, afford neuroprotection in vivo, as previously shown in models of cerebral ischemia, excitotoxicity and devascularization-induced neurodegeneration, and (2) support a role of the locus coeruleus noradrenergic system in promoting survival of neurons in areas of the brain where neurogenesis persists in the adult.

Keywords: Neurogenesis; Proliferation; Dexefaroxan; F 14413; Activated glia; SEL-OB; Noradrenaline

Introduction

Dysfunction of the noradrenergic system has been suggested to contribute to progressive brain damage in neurodegenerative disorders (Colpaert, 1994; Friedman et al., 1999; Heneka et al., 2002; Klotz et al., 2003; Marien et al., 2004). Extensive losses of LC-noradrenergic neurons occur in Alzheimer's and Parkinson's diseases (Lyness et al., 2003; Zarow et al., 2003), suggesting that a disruption of the noradrenergic afferent inputs to the olfactory bulb (OB) might contribute to the structural alterations (Kovacs et al., 2001) and functional olfactory deficits (Bacon et al., 1998; Devanand et al., 2000; Mesholam et al., 1998) that occur early in these diseases.

Abbreviations: AOB, accessory olfactory bulb; BDNF, brain-derived neurotrophic factor; BSA, bovine serum albumin; CNS, central nervous system; BrdU, bromodeoxyuridine; dUTP; 2′-deoxyuridine-5′-triphosphate; DAB, diaminobenzidine; DNA, deoxyribonucleic acid; EPL, external plexiform layer; FITC, fluorescein isothiocyanate; GFAP, glial fibrillary acidic protein; GL, glomerular layer; GCL, granule cell layer; HPLC, high performance liquid chromatography; LC, locus coeruleus; NA, noradrenaline; NeuN, neuronal-specific antigen; OB, olfactory bulb; 6-OHDA, 6-hydroxydopamine; ONL, olfactory nerve layer; PBS, phosphate-buffered saline; RMS, rostral migratory stream; SEL, subependymal layer; SVZ, subventricular zone; Tdt, terminal dideoxynucleotidyl transferase; TRIS, Tris(hydroxymethyl)aminomethane; TUNEL, terminal dideoxynucleotidyl transferase-catalyzed 2′-deoxyuridine-5′-triphosphate nick end-labeling.

* Corresponding author. Laboratoire de Neurosciences et Systèmes Sensoriels, CNRS-UMR 5020, Université Claude Bernard-Lyon 1, 50 Avenue Tony Garnier, F-69366 Lyon, France. Fax: +33 4 37 28 76 01.
E-mail address: jourdan@olfac.univ-lyon1.fr (F. Jourdan).

0014-4886/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.expneurol.2005.03.004

The OB is the first central relay in the olfactory sensory pathway. This paleocortex is the only known target of neural progenitors produced throughout life in the subventricular zone (SVZ) of the forebrain (Luskin, 1993). Progenitors migrate along the rostral migratory stream (RMS) (Lois and Alvarez-Buylla, 1994; Peretto et al., 1999) and reach the subependymal layer of the OB (SEL-OB) from where they migrate radially and differentiate into granular and periglomerular interneurons (Belluzzi et al., 2003; Carleton et al., 2003; Petreanu and Alvarez-Buylla, 2002). A proportion of newly formed neurons die early after differentiation in the OB (Petreanu and Alvarez-Buylla, 2002; Winner et al., 2002), whereas others survive and may contribute to local processing related to olfactory discrimination (Gheusi et al., 2000) and memory (Rochefort et al., 2002).

We have previously demonstrated that spontaneous neuronal death in the OB was reduced by a 7-day treatment with dexefaroxan (Bauer et al., 2003), a centrally active α_2-adrenoceptor antagonist (Mayer et al., 2001). This neuroprotective effect was suggested to be due in part to a facilitation of noradrenaline (NA) release from the rich afferent input to the OB that originates in the locus coeruleus (LC) (McLean et al., 1989), by a blockade of the inhibitory presynaptic α_2-autoreceptors on LC-noradrenergic afferents that regulate cortical NA release in vivo (Dennis et al., 1987). The aim of the present study was to extend our previous investigations, by examining the effects of α_2-adrenoceptor blockade on neurogenesis in the situation of a surgical deafferentation (olfactory axotomy) where neuronal death and cell proliferation are actively upregulated as a result of this injury (Mandairon et al., 2003). Dexefaroxan, and a new α_2-adrenoceptor antagonist of a different chemical series, F 14413 ([+]-5-fluoro-methoxyidazoxan) (Mayer et al., 2003), were both evaluated in a model where a unilateral axotomy procedure was previously shown to modify cell death and proliferation in the OB of adult mice at 6–8 days after surgery (Mandairon et al., 2003).

Materials and methods

Animals

Animals were handled and cared for in accordance with the European Community Council Directive of November 24th 1986 (86/609/ECC). The experimental protocols were carried out in compliance with local ethical committee guidelines for animal research. Eighty male C57BL/6J mice (Charles River Laboratories, Domaine des Oncins, France), aged 8–12 weeks at the beginning of the experiment, were used. Animals had free access to water and food and were housed 4–6 per cage at 20–24°C under a 12:12-h light/dark cycle (lights on at 7:00 h).

Surgery

Unilateral olfactory nerve axotomy was performed as previously described (Mandairon et al., 2003). Briefly, animals were anesthetized with Equithesine (0.2 ml/100 g body weight ip). A longitudinal incision was made in the skin overlying the skull and a craniotomy was performed over the OB on one side. In the animals subjected to the axotomy (n = 30), olfactory nerve bundles were surgically severed with a small lancet at the anterior tip of the OB abutting against the cribiform plate (Fig. 1). The skin was then sutured and after recovery from anesthesia the animals

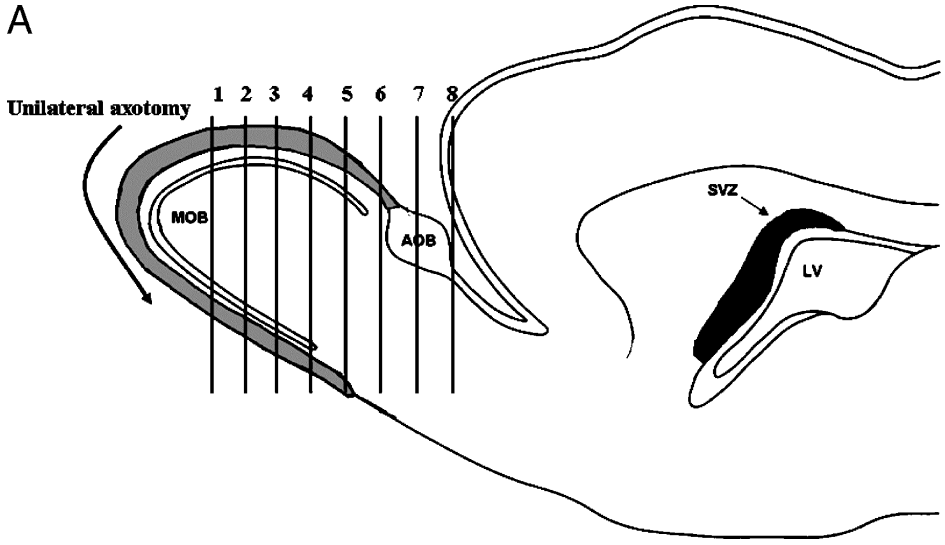

Fig. 1. Schematic sagittal representation of the anterior part of the mouse brain. Unilateral olfactory axotomy was performed by severing the olfactory nerve axons at the anterior tip of the olfactory bulb (OB) (arrow). Cell counts in the OB were made on 6 coronal sections (14 μm thick) separated by 336 μm intervals. The sampling covered the main olfactory bulb (MOB), from its anterior tip to the rostral apex of the accessory olfactory bulb (AOB).