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Research Report

Neurotrophin expression in the adult olfactory epithelium

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ABSTRACT

Published reports of neurotrophin expression in the olfactory system are incomplete because of missing data and conflicting results. Previous studies used a variety of fixation procedures and antibodies on different species and different ages. The aim of the present study was to examine expression of neurotrophins and their receptors using optimized methodologies: five methods of fixation, multiple antibodies, a variety of immunochemical protocols, and RT-PCR. We show here that (i) transcripts for all neurotrophins and their receptors are found in the adult olfactory epithelium; (ii) all neurotrophins are expressed in the supporting cells and the neuronal layers of the undisturbed adult olfactory epithelium while NT4 is found additionally in the horizontal basal cells; (iii) neurotrophin immunoreactivity required a fixative that included parabenzoquinone (not used in previous studies of olfactory tissue); (iv) TrkB and TrkC are restricted to the globose basal cell and neuron layers while TrkA is found in the horizontal basal cells and in the supporting cells where it co-localizes with the low affinity receptor for NGF (p75NTR). These findings confirm that neurotrophins are produced within the olfactory epithelium, suggesting autocrine and paracrine regulation of olfactory neurogenesis.

1. Introduction

The neurogenesis that occurs throughout life in the olfactory mucosa (Breipohl et al., 1986; Graziadei and Monti Graziadei, 1978; Mackay-Sim and Chuah, 2000), including in humans (Murrel et al., 1996), is a delicate balance between cellular proliferation, migration, differentiation, survival and death. Over the recent years a multitude of growth factors have been identified in the peripheral olfactory system, often acting on specific cell types (Mackay-Sim and Chuah, 2000). Neurotrophins and their receptors are part of this complex and interactive network.

Neurotrophins constitute a family of polypeptide growth factors that include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and neurotrophin 4/5 (NT4/5) (Bothwell, 1995). They exert their biological functions by binding to high affinity transmembrane receptors belonging to the Trk family of tyrosine kinase receptors (Barbacid, 1995). The preferred receptor for each neurotrophin is: TrkA for NGF, TrkB for BDNF and NT4/5, TrkC for NT3 (Maness et al., 1994). In addition, all neurotrophins bind to the glycoprotein $p75^{NTR}$ that belongs to the super family of tumor necrosis factor (TNF) receptors (Chao, 1994).

Numerous studies have documented the expression of neurotrophins and their receptors in the olfactory epithelium (Carter and Roskams, 2002; Mackay-Sim and Chuah, 2000). NGF was found in the neurons of the embryonic olfactory

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mucosa (Ayer-LeLievre et al., 1983; Williams and Rush, 1988) and in clusters of neurons in 3-week-old rats (Aiba et al., 1993) but was not detected in olfactory epithelium in adult rats (Roskams et al., 1996). During regeneration of the olfactory sensory neurons in adult, the regenerating axons exhibited NGF immunoreactivity (Roskams et al., 1996). BDNF was found in horizontal basal cells (Buckland and Cunningham, 1999). NT3 has been reported as absent (Deckner et al., 1993; Guthrie and Gall, 1991) or present in supporting cells (Simpson et al., 2003) and in a discrete subset of mature neurons (Vigers et al., 2003). Olfactory ensheathing cells are a specialized glial cell surrounding the olfactory sensory axons as they leave the epithelium. These cells express NGF, BDNF and all neurotrophin receptors (Bianco et al., 2004; Boruch et al., 2001; Lipson et al., 2003; Woodhall et al., 2001).

TrkA was reported present in a restricted population of horizontal basal cells in the normal olfactory epithelium and by all horizontal basal cells in the regenerating epithelium (Miwa et al., 1998; Roskams et al., 1996). TrkA was also found in (i) the dendrites and axon bundles in normal and axotomized rat olfactory receptor neurons (Yasuno et al., 2000) and (ii) the somata of the zebrafish crypt sensory neurons (Catania et al., 2003). TrkB and TrkC immunoreactivity was observed on developing (Holcomb et al., 1995) and adult neurons (Deckner et al., 1993; Nibu et al., 1999; Roskams et al., 1996). Studies of $p75^{NTR}$ expression are in conflict. In several studies it was reported to be restricted to the olfactory ensheathing cells in the lamina propria (Gong et al., 1994; Roskams et al., 1996; Vickland et al., 1991) but three other studies reported its expression in the regenerating (Miwa et al., 1993; Turner and Perez-Polo, 1992) and normal olfactory epithelium (Aiba et al., 1993).

Such discrepancies could be explained by disparities in the use of species, genders and lifetime points. However, we hypothesized that the contradictions in the literature may have arisen because of the variety and insufficiency of methods used by previous authors. For example, neurotrophins are best preserved with a fixative containing parabenzoquinone (Conner, 2001) and peptides often require non-denaturing fixation (Bu'Lock et al., 1982). The choice of antibodies is another difference between published studies. The aim of the study was to provide a comprehensive analysis of neurotrophin and neurotrophin receptor expression in the adult olfactory epithelium using a variety of fixation methods and antibodies. The results were confirmed by analysis of mRNA expression using RT-PCR on purified olfactory epithelium, according to a technique devised for *in vitro* experiments (Féron et al., 1999).

2. Results

2.1. *All neurotrophins were present in the olfactory epithelium*

Fig. 1 shows that all neurotrophins were observed in the olfactory epithelium after optimizing the methods (Table 1). NT4 was mainly expressed around the nucleus among all cells (Fig. 1D). NGF was observed in all cell layers except the horizontal basal cells. NGF was expressed throughout dendrites and axons of neurons and within the apical cytoplasm of the supporting cells (Fig. 1A). BDNF and NT3 expression patterns were similar to NGF being mainly found in the upper two thirds of the epithelium with an overall perinuclear location (Figs. 1B, C).

2.2. *All Trk receptors and p75 were present in the olfactory epithelium*

As shown in Fig. 1, all neurotrophin receptors were observed in the olfactory epithelium under optimized conditions (Table 1). TrkB and TrkC were expressed in cell layers containing neuronal progenitors, immature neurons and mature sensory neurons. Both receptors were present in the perinuclear region but only TrkB was expressed along the dendrites (Figs. 1F, G). TrkA was expressed by non-neuronal cell types, supporting cells and horizontal basal cells (Fig. 1E). $p75^{NTR}$ expression was restricted to a subset of supporting cells and some rare neurons (Fig. 1H).

2.3. *A parabenzoquinone-containing fixative was essential for neurotrophin immuno-detection*

Sections of trigeminal nerves and dorsal root ganglia were used for defining optimal fixation and immunostaining procedures (data not shown). The main results are summarized in Table 1. Addition of parabenzoquinone to a light (2%) paraformaldehyde fixative was necessary for obtaining robust immunostaining of the neurotrophins in the olfactory epithelium. In most cases, the Santa Cruz antibodies provided the strongest staining, with the advantage of having a specific blocking peptide to confirm specificity. For $p75^{NTR}$ the better antibody was obtained from Neubody (Australia) combined with an antigen retrieval procedure while avoiding permeabilization steps.

2.4. *Neurotrophins and receptor mRNA expression*

Brain mRNA was used for optimizing PCR conditions (data not shown). As demonstrated in Fig. 2, transcripts of all neurotrophins and neurotrophin receptors were found in the olfactory epithelium.

3. Discussion

We show here that the neurotrophins (NGF, BDNF, NT3, and NT4) and their receptors (TrkA, TrkB, TrkC, and $p75^{NTR}$) are expressed throughout the adult olfactory epithelium, summarized in Fig. 3. Their expression in the olfactory epithelium was observed using immunohistochemistry and confirmed with RT-PCR, illustrating that all neurotrophins were made locally, without requiring retrograde axonal transport. NT4 appears to be produced by every cell type, including horizontal basal cells, whereas the other three neurotrophins were not expressed in the deepest layers of the epithelium. Similarly, the receptors TrkB and TrkC were present on neural progenitors and sensory neurons only whereas TrkA and $p75^{NTR}$ were not present on these cells but rather only on horizontal basal cells and supporting cells. These results suggest autocrine and