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Research Report

A comparative examination of biomarkers for olfactory ensheathing cells in cats and guinea pigs

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ABSTRACT

We investigated the neurochemical characteristics of olfactory ensheathing cells (OECs) in adult cats and in adult guinea pigs. Three conventional biomarkers for OECs, p75 neurotrophin receptor (p75NTR), S100, and glial fibrillary acidic protein (GFAP), as well as two recently identified biomarkers, smooth muscle α-actin (SMA) and calponin, were used. We found that 1) antibodies against SMA and S100 yielded positive immunostaining of mucosal and bulbar OECs in cats and guinea pigs; 2) antibodies against GFAP gave positive immunostaining of mucosal and bulbar OECs in cats; and 3) antibodies against calponin yielded positive immunostaining of bulbar OECs in adult cats. Unexpectedly, antibodies against p75NTR failed to positively stain mucosal and bulbar OECs in cats and guinea pigs, and antibodies against GFAP and calponin failed to positively stain mucosal and bulbar OECs in guinea pigs. These findings show the importance for empirical testing of all biomarkers for OECs among different mammalian species when attempting to identify these cells *in vivo*, *in vitro*, and following intraspinal implantation.

1. Introduction

The mammalian olfactory nervous system consists of the olfactory mucosa and the olfactory bulbs. The olfactory mucosa, located in the posterior nasal cavity, is composed of two layers: a superficial avascular layer of pseudostratified columnar neuroepithelium, and a deeper highly vascularized lamina propria (Margolis and Getchell, 1988). The cell bodies of the olfactory neurons and their basal stem cells are situated in the neuroepithelium. Small diameter (0.1–0.7 μm) unmyelinated olfactory axons of these neurons penetrate the underlying lamina propria where they become tightly bundled together by the olfactory ensheathing cells (OECs). OECs ensheath several hundred olfactory axons and together create olfactory nerve fascicles. Each olfactory nerve fascicle is bound by an outer layer of flattened fibroblast-like cells, which are often referred to as olfactory (or perineurial) nerve fibroblasts. In addition to fascicles of OECs and olfactory axons, the lamina propria has small arterioles and an extensive venous plexus, as well as peripheral nerves that carry sensory and autonomic fibres. As the olfactory nerve fascicles approach the cribriform plate, they coalesce into even larger bundles that become intermeshed in the olfactory nerve fiber layer of the olfactory bulbs. Here the fascicular association between OECs and olfactory axons is replaced by clusters of OECs extending processes around the crisscrossing bundles of olfactory axons. The olfactory axons finally penetrate into the olfactory bulb where they innervate the dendritic processes of mitral, periglomerular, and tufted neurons in the glomerular layer (Valverde et al., 1992).

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To date, numerous research groups have shown that cultures containing OECs can be obtained from the lamina propria of the olfactory mucosa and/or the olfactory nerve fiber layer of the olfactory bulb (Au and Roskams, 2003; Ramon-Cueto et al., 1993; Rubio et al., 2008). Though mucosal and bulbar OECs have the same developmental origin (Rubio et al., 2008) and were once considered to be identical (Chuah and Au, 1991; Doucette, 1993), it appears that mucosal and bulbar OECs can display a variety of cellular morphologies and phenotypic properties *in vitro*. One consistent observation is that all cultured OECs, regardless of the olfactory source, age of the donor, or species, exhibit positive immunostaining for three biomarkers: p75 neurotrophin receptor (p75NTR), S100, and glial fibrillary acidic protein (GFAP) (Barber and Lindsay, 1982; Devon and Doucette, 1992; Gong et al., 1994). Our laboratory has shown that OECs from embryonic and adult rats also express smooth muscle α-actin (SMA), as well as a variety of other actin-binding proteins such as calponin, both *in vivo* and *in vitro* (Boyd et al., 2006; Jahed et al., 2007; Kawaja et al., 2009; c.f. Ibanez et al., 2007; Tome et al., 2007).

In recent years, there has been a growing interest in assessing OECs *in situ* and *in vitro* from a number of different mammalian species, including rats, mice, dogs, pigs, and non-human primates. Our laboratory has recently shown several interesting ultrastructural differences (i.e., the fascicular arrangement between OECs and olfactory axons) between mucosal and bulbar OECs from adult mice, rats, and cats (Kawaja et al., 2009). Might similar differences be seen when comparing the neurochemical phenotypes of OEC in the olfactory mucosa and olfactory bulbs of adult mammals? In this study we undertook a comparative examination of biomarkers that may be used to identify OECs in the olfactory tissues of adult cats and adult guinea pigs. Here we show that while markers such as S100 and SMA can be used to reliably localize mucosal and bulbar OECs in adult cats and adult guinea pigs, markers such as p75NTR, GFAP, and calponin yield inconsistent immunostaining for these olfactory glia. Consideration of these new observations should be given in future studies that will examine the neurochemical phenotype of OECs isolated from other mammalian species, including dogs, pigs, and non-human primates.

2. Results

2.1. *Ultrastructural characteristics of feline and guinea pig OECs*

We have recently documented the ultrastructural features of mucosal and bulbar OECs and their association with olfactory axons in adult mice, rats, and cats (Kawaja et al., 2009). Curiously, each of these aforementioned species displays a slightly different anatomical arrangement of OECs and olfactory axons within the olfactory nerve fascicles of the mucosa. Here we offer new information regarding OECs in the adult guinea pig. In longitudinal views of the olfactory nerve fascicles, mucosal OECs in adult guinea pigs bear a striking resemblance to OECs found in adult mice, such that several OECs are seen extending processes that together ensheath the olfactory axons within a single nerve fascicle (Fig. 1A). By contrast, single OECs in the feline olfactory nerve fascicles are seen enveloping numerous olfactory axons (cross-sectional views in Figs. 1B, C). These OEC-axon units are bound by basal lamina, and collagen fibrils are found in the spaces between these OEC-axons units. In both species, flattened olfactory nerve fibroblasts form the outer limits of the olfactory nerve fascicles. There are two additional points worth mentioning. First, most olfactory nerve fascicles in the mucosa of adult cats have a dedicated arteriole that passes amongst the OEC-axon units; such intrafascicular arterioles are not seen in adult mice, or rats. Second, the mucosa of mice, rats, cats, and guinea pigs have peripheral nerves containing Schwann cells, which can often be found in close proximity to the olfactory nerve fascicles.

As for bulbar OECs, both cats and guinea pigs (like mice and rats) loose their mucosal appearance, such that these cells are found in clusters within the olfactory nerve fiber layer. Rather than forming an elaborate ensheathment of olfactory axons, bulbar OECs extend numerous processes that collectively envelop olfactory axons into very large bundles that crisscross each other as the fibers penetrate into the olfactory bulb (data not shown).

2.2. *Immunolocalization of SMA*

In the olfactory mucosa of adult cats and adult guinea pigs, SMA immunostaining was found in a variety of cell types including vascular smooth muscle cells (VSMCs) of arterioles located within the nerve fascicles, perineurial fibroblasts, and OECs (Figs. 2A–C). As per suggested by the manufacturer, tissue may be treated with a protease to reveal SMA-immunoreactivity. SMA-immunopositive VSMCs and perineurial fibroblasts were readily seen in tissues without the aid of proteinase K. Only following treatment with proteinase K were SMA-immunopositive OECs revealed within the feline olfactory mucosa and in the olfactory nerve fiber layer of the olfactory bulb (Figs. 2A, D). No protease (e.g., proteinase K) treatment, however, was required to reveal SMA-immunopositive OECs within the olfactory mucosa or olfactory bulb of adult guinea pigs (Figs. 2B, C, E). In the lamina propria of adult cats and guinea pigs, SMA-immunopositive mucosal OECs were identified as those cells having multiple processes within the olfactory nerve fascicles; by comparison, SMA-immunopositive perineurial fibroblasts were identified as those flattened cells surrounding the nerve fascicles. We have described a similar staining pattern in the olfactory mucosa of adult rats (Jahed et al., 2007; Kawaja et al., 2009). In the olfactory bulbs of adult cats and adult guinea pigs, clusters of SMA-immunopositive OECs were found in the olfactory nerve fiber layer (Fig. 2D, E). In the deeper layers of the feline and guinea pig olfactory bulbs, cells that appear to be astrocytes displayed positive immunostaining for SMA as well. These latter results concur with a previous report by Lecain et al. (1991) who showed SMA-immunopositive astrocytes in adult mice.

2.3. *Immunolocalization of S100*

As seen for SMA-immunopositive staining, VSMCs, perineurial fibroblasts, and OECs in the olfactory mucosa of adult cats