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Research Report

# Spatio-temporal expression pattern of receptors for myelin-associated inhibitors in the developing rat olfactory system

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ABSTRACT

The myelin-associated inhibitory proteins (Nogo-A, MAG and OMgp) that prevent axon regeneration in adult CNS, mediate their effects via a receptor referred as NgR1. Beside their inhibitory role in the adult CNS, Nogo-A and NgR1 might also be functionally involved in the developing nervous system. At the present time, no detailed study is available regarding either the onset of NgR1 expression during development or its spatio-temporal pattern of expression relative to the presence of Nogo-A. Two homologs of NgR1, NgR2 and NgR3, have been recently identified, but their function in the nervous system is still unknown in adult as well as during development. We have examined the spatio-temporal expression pattern of both NgR1, NgR2 and NgR3 mRNAs and corresponding proteins in the developing rat olfactory system using in situ hybridization and immunohistochemistry. From E15–E16 onwards, NgR1 mRNA was expressed by differentiating neurons in both the olfactory epithelium and the olfactory bulb. At all developmental stages, including adult animals, NgR1 protein was preferentially targeted to olfactory axons emerging from the olfactory epithelium. Using double-immunostainings in the postnatal olfactory mucosa, we confirm the neuronal localization of NgR1 and its preferential distribution along the olfactory axons. The NgR2 and NgR3 transcripts and their proteins display similar expression profiles in the olfactory system. Together, our data suggest that, in non-pathological conditions, NgR1 and its homologs may play a role in axon outgrowth in the rat olfactory system and may be relevant for the confinement of neural projections within the developing olfactory bulb.



## 1. Introduction

Nogo-A, MAG (myelin-associated glycoprotein) and OMgp (oligodendrocyte-myelin glycoprotein) are myelin-associated inhibitory proteins that prevent axon regeneration in the lesioned central nervous system (CNS) of adult animals (Filbin, 2003; Schwab, 2004; Liu et al., 2006; Xie and Zheng, 2008). Although structurally dissimilar, these proteins mediate their

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*Abbreviations:* E1, Embryonic day 1; GAP43, Growth-Associated Protein of 43 kDa; MAP2, Microtubule-Associated Protein 2; NgR, Nogo-66 Receptor; OB, Olfactory Bulb; OE, Olfactory Epithelium; OMP, Olfactory Marker Protein; ORN, Olfactory Receptor Neuron; P1, Postnatal day 1

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effects via a common neuronal receptor, the Nogo-66 receptor also referred as NgR1 (Fournier et al., 2001; Domeniconi et al., 2002; Liu et al., 2002; Wang et al., 2002b). NgR1 is a member of the proteoglycan/leucine-rich repeat protein family and is attached to the cell surface by a C-terminal glycosyl-phosphatidylinositol (GPI)-anchor (Fournier et al., 2002; He et al., 2003; Pignot et al., 2003; Yu et al., 2004) (see also Schwab et al., 2006 and Giger et al., 2008 for reviews). In order to transduce the growth inhibitory signals across the cell membrane, NgR1 associates with several signaling partners, namely the p75 neurotrophin receptor ($p75^{NTR}$) or TROY receptor and LINGO-1, a transmembrane brain-specific protein (Wang et al., 2002a; Wong et al., 2002; Mi et al., 2004; Park et al., 2005; Shao et al., 2005). The binding of myelin-associated inhibitory proteins to the tripartite receptor complex induces the activation of the Rho GTPases pathway, resulting in the rearrangement of the actin cytoskeleton and thus leading to growth cone collapse (Niederost et al., 2002) (see also Yiu and He, 2003 for review).

In the mature brain, NgR1 mRNA expression is restricted to specific sets of neurons (Hunt et al., 2002b; Josephson et al., 2002; Mingorance et al., 2004; Hasegawa et al., 2005; Barrette et al., 2007; Funahashi et al., 2008). NgR1 protein is mainly detected in neuronal cell bodies and to a lesser extent in neuritic processes (Wang et al., 2002c). There is a general agreement that Nogo-A/NgR1 interaction constitutes one of the major impediments to axonal regeneration in the injured CNS (Filbin, 2003; Schwab, 2004; Liu et al., 2006; Xie and Zheng, 2008). Nevertheless, several data suggest that Nogo-A/NgR1 may also have a physiological function in the intact adult CNS, unrelated to injury or regeneration. Notably, it has been shown that Nogo-A/NgR1 interaction may stabilize mature myelinated fiber tracts by preventing unnecessary axonal sprouting, thus contributing to the maintenance of specific connections (Wang et al., 2002c; Raisman, 2004; McGee et al., 2005).

Beside these functions in the adult CNS, it becomes more and more obvious that Nogo-A and NgR1 might also play a role in the developing nervous system. NgR1 mRNA is expressed during the nervous system development from late embryonic stages in fish, rodent and human (Hunt et al., 2002b; Josephson et al., 2002; Klinger et al., 2004). Both Nogo-A and NgR1 transcripts have been detected in neocortical neurons, in hippocampal pyramidal neurons and in motor neurons of the spinal cord in several species (Josephson et al., 2001; Josephson et al., 2002; Mingorance et al., 2004). By contrast, little information is available regarding the expression patterns of Nogo-A and NgR1 proteins in the developing nervous system. Several studies have shown that Nogo-A protein is present in some neuronal populations during brain development, with a preferential distribution within outgrowing axons (Huber et al., 2002; Tozaki et al., 2002; Wang et al., 2002c; Mingorance et al., 2004; Mingorance-Le Meur et al., 2007). However, no detailed study is yet available about the onset of NgR1 expression in the developing nervous system and its spatio-temporal distribution relative to the presence of Nogo-A. We recently reported that Nogo-A is detected within outgrowing olfactory axons, at both developmental and adult stages, supporting a role of this protein in axon outgrowth in the intact nervous system (Richard et al., 2005). In order to determine whether the NgR1 receptor might be involved in Nogo-A signaling in the developing olfactory system, we study here the spatio-temporal pattern of expression of both NgR1 mRNA and protein in this system. We also describe the expression profiles of two newly identified members of the NgR family, NgR2 and NgR3, that share common structural features with NgR1 (Barton et al., 2003; Lauren et al., 2003; Pignot et al., 2003). Our results show that NgR1, NgR2 and NgR3 mRNAs and proteins display similar neuronal expression patterns in the developing olfactory system, with a preferential targeting of NgR proteins to olfactory axons.

## 2. Results

### 2.1. *Spatio-temporal profile of NgR1 expression in the developing olfactory system*

#### 2.1.1. *In situ hybridization data*

The first NgR1 mRNA expression in the olfactory system was detected in E16 embryos. NgR1 mRNA appeared weakly expressed by some differentiating cell bodies located in the olfactory epithelium and in the marginal zone of the developing olfactory bulb (Fig. 1A). Note that no signal was detected in the dividing progenitor cells from the ventricular zone. In E16 embryos as in later embryonic stages, no signal was observed in adjacent sections hybridized with the sense probe (Fig. 1B). From E18 (Fig. 1C) to E21 (Fig. 1D), numerous NgR1 mRNA-positive cells were present in both the olfactory and vomeronasal epithelia. As shown in Fig. 1G, a strong accumulation of NgR1 transcript was also detected in the presumptive mitral cell layer of the olfactory bulb in E21 embryos.

In young postnatal rats (P1–P6), a sustained NgR1 mRNA expression was observed in most neuronal cell bodies distributed throughout the thickness of the olfactory and vomeronasal epithelia (Fig. 1E). In the olfactory bulb, a strong staining for NgR1 mRNA was displayed by the mitral cell bodies (Fig. 1H). In addition, a faint signal was also detected at P6 in periglomerular or tufted cells located on the internal side of the glomeruli (Fig. 1H).

In P15 and P40 rats, a weak to moderate NgR1 mRNA expression was observed in cell bodies widely distributed in the whole thickness of the epithelium (Fig. 1F). Considering the number and the distribution of the labeled cell bodies within the epithelium, we can assume that most olfactory receptor neurons (ORNs) express NgR1 mRNA, including mature ORNs located in the middle part of the epithelium (Farbman and Margolis, 1980; Schwob, 2002). No specific signal was detected in the adjacent sections hybridized with the sense probe, thus confirming the specificity of the labeling (Fig. 1F'). In the adult olfactory bulb, the pattern of NgR1 mRNA expression was similar to that reported in younger rats, although the hybridization signal seemed less intense (Fig. 1I).

#### 2.1.2. *Immunohistochemical data*

The immunohistochemical analysis was carried out on sections of the olfactory epithelium and olfactory bulb by using two different polyclonal anti-NgR1 antisera. Since similar results were obtained with both antibodies, we decided to present only data collected with the anti-NgR1 antibody, which has been previously characterized and validated (Venkatesh et al., 2005).