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Neuronal nitric oxide synthase (nNOS) immunoreactivity in the olfactory system of a cartilaginous fish

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ABSTRACT

Nitric oxide is a regulative molecule with important roles in the olfactory system of vertebrates. Chondrichtyans have a key position in vertebrate evolution and nothing is known about nitric oxide in their olfactory system. Aim of this work was to investigate the neuronal nitric oxide synthase (nNOS) immunoreactivity in the olfactory system of the shark *Scyliorhinus canicula*. Because nitric oxide is often related to GABA in the olfactory system, also the distribution of GABA and its synthesis enzyme GAD has been investigated. In the olfactory epithelium scattered cells in the basal and medial zone of the epithelium thickness presented nNOS-like immunoreactivity. In the olfactory bulb the nNOS-like immunoreactivity has been highlighted in nerve fibers around some blood vessels and in scattered GABAergic granule cells.

The presence of nNOS in the olfactory system of *S. canicula* is overall lesser than that described in other vertebrates, even if nitric oxide probably keeps some essential functions.

1. Introduction

Two systems are devoted to olfaction in vertebrates: the olfactory and the vomeronasal ones. In tetrapod, they are anatomically distinct while, in fish, they are morphologically lodged in the same organs, and they are commonly called "olfactory system" even if they bear both olfactory and vomeronasal features (e.g. Hansen et al., 2003).

Compared to the olfactory system of other fishes, the olfactory system of Chondrichtyans is characterized by some peculiar lacks: their olfactory epithelium (OE) lacks of ciliated sensory neurons (Holl, 1973; Theisen et al., 1986; Franceschini and Ciani, 1993; Takami et al., 1994); in their olfactory bulb (OB), the periglomerular cells are absent or rare (Rodríguez-Moldes et al., 1993; Dryer and Graziadei, 1996); the two main olfactory receptor families, olfactory receptors (ORs) and trace-amino associated receptors (TAARs), are almost missing in the genome of the elephant shark *Callorhinchus milii* (Grus and Zhang, 2009; Niimura, 2009); immunohistochemical detection of the olfactory-type G protein alpha subunit (a marker of the olfactory system) always failed to find any immunoreactivity (ir) in the olfactory system of investigated species (Ferrando et al., 2009, 2010a).

Nitric oxide (NO) is a gas that in living organisms acts as a messenger and is likely to have important roles in olfaction, such as the regulation of neuronal precursor proliferation and synaptic maintenance (Chen et al., 2004), memory consolidation (Jüch et al., 2009) and regulation of neurotransmitters release or uptake (Crespo et al., 2003). NO is synthesized from arginine by the enzymes NO synthases (NOS) and, in particular in the nervous system, by the neural NOS (nNOS) (Garthwaite, 2008). Although the fundamental role of NO in the olfactory system, the distribution of nNOS in the olfactory and vomeronasal systems of vertebrates is quite variable.

In mammals, nNOS is present in the OB (in granule cells, cells of the plexiform layer, and weakly, in some periglomerular cells) and in the vomeronasal accessory OB (AOB) (in granule cells) (Kishimoto et al., 1993; Iwase et al., 1998; Matsuda et al., 1996; Kosaka and Kosaka, 2007b; Wenisch and Arnhold, 2010). Few data are reported in the literature about reptilians and birds: immunohistochemistry and histoenzymatic reaction for NOS, show the absence of such enzyme from the olfactory and vomeronasal systems (Brüning et al., 1994a,b; Jiang and Terashima, 1996). In adult amphibian, nNOS ir has been observed in the OB (in granule cells) and in the anuran AOB (in granule cells) (Porteros et al., 1996). A peculiar distribution has been found in the neotenic adult *Ambystoma mexicanum*, where nNOS ir has been detected in sensory receptor neurons and some basal cells (BCs) of both olfactory and vomeronasal epithelium (Sánchez-Islas and

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León-Olea, 2001). In teleost fish nNOS ir is described in the OE (in olfactory receptor neurons and in some BCs), in the OB (in some granule cells) in *Oreochromis mossambicus* (Singru et al., 2003) but not in other species (Giraldez-Perez et al., 2008; Gaikwad et al., 2009).

In sea lamprey, nNOS-like ir has been described in the OE of larvae (mainly in BCs) and adult (mainly in olfactory receptor neurons) (Hua et al., 2000).

No data are present in the literature about the nNOS distribution in the peripheral olfactory system in the class of Chondrichtyans, and it deserves investigation also in the light of the peculiar features described above.

In the brain, the nitrergic system is integrated with other systems of neurotransmission. Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system of vertebrates (Siegel et al., 1999) and in the olfactory system as well (Gire and Schoppa, 2009). It has been demonstrated that NO enhances the basal GABA release from GABAergic elements in some brain areas and the presence of GABAergic interneurons containing nNOS has been reported in the OB of mammals (Crespo et al., 2003).

The aim of this study is to be a base for the investigation of the nitrergic system in the olfactory organs of the shark *S. canicula*, describing for the first time, the distribution of nNOS ir, giving also a first insight in the relation between the nitrergic and the GABAergic system in the olfactory system of this species.

2. Materials and methods

Six adult specimens (4 males and 2 females) of *S. canicula*, with lengths ranging from 48 to 53 cm, were collected in the Irish Sea (NE Atlantic). Three specimens

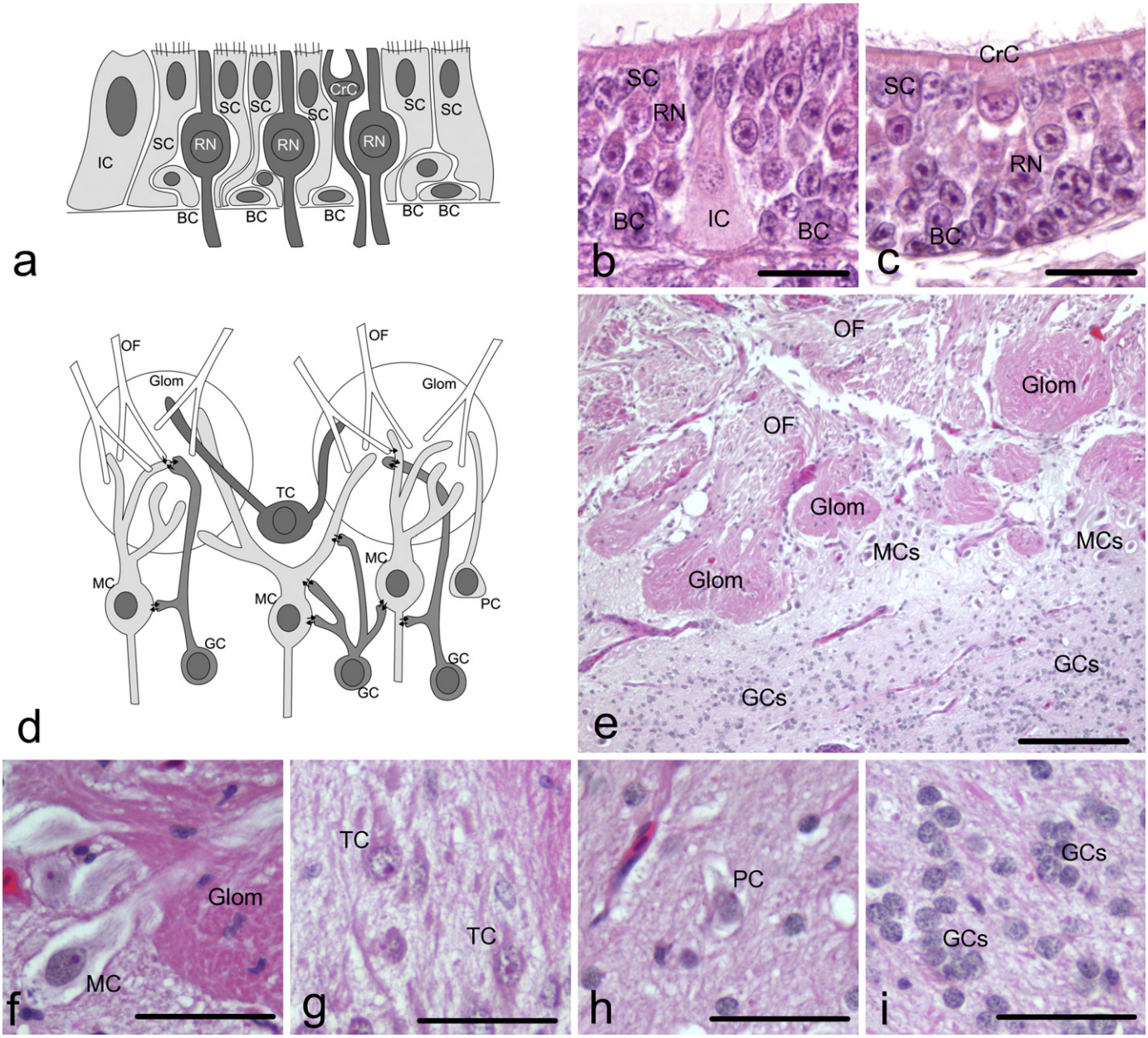

Fig. 1. (a–c) Olfactory epithelium of *S. canicula*. (a) Scheme of different cell types. (b and c) Hematoxylin–eosin stained histological section. Scale bars 20 μm. (d–i) Olfactory bulb of *S. canicula*. (d) Scheme of different cell types and fibers in the olfactory bulb of *S. canicula*. The cytoarchitectonic and the synaptic contacts are those reported in the literature for elasmobranches (Sterzi, 1909; Rodríguez-Moldes et al., 1993; Dryer and Graziadei, 1996; Anadón et al., 2000). (e–i) Hematoxylin–eosin stained histological section. (e) Scale bar 320 μm. (f–i) Scale bars 50 μm. BC = basal cell; CrC = crypt cell; GC = granule cell; Glom = glomerulus; IC = ionocyte; MC = mitral cell; OF = olfactory fiber; PC = periglomerular-like cell; RN = receptor neuron; SC = sustaining cell; TC = tufted-like cell.