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Development of the serotonergic system in the central nervous system of the sea lamprey☆

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Abstract

Lampreys belong to the most primitive extant group of vertebrates, the Agnathans, which is considered the sister group of jawed vertebrates. Accordingly, characterization of neuronal groups and their development appears useful for understanding early evolution of the nervous system in vertebrates. Here, the development of the serotonergic system in the central nervous system of the sea lamprey, *Petromyzon marinus*, was investigated by immunohistochemical analysis of specimens ranging from embryos to adults. The different serotonin-immunoreactive (5-HT-ir) neuronal populations that are found in adults were observed between the embryonic and metamorphic stages. The earliest serotonergic neurons were observed in the basal plate of the isthmus region of late embryos. In prolarvae, progressive appearance of new serotonergic cell groups was observed: firstly in the spinal cord, then in the pineal organ, tuberal region, zona limitans intrathalamica, rostral isthmus, and the caudal part of the rhombencephalon. In early larvae a new group of serotonergic cells was observed in the mammillary region, whereas in the pretectal region and the parapineal organ the first serotonergic cells were seen in the middle and late larval stages, respectively. The first serotonergic fibres appeared in early prolarvae, with fibres that ascend and descend from the isthmic cell group, and the number of immunoreactive fibres increased progressively until the adult stage. The results reveal strong resemblances between lampreys and other vertebrates in the spatio-temporal pattern of development of brainstem populations. This study also reveals a shared pattern of early ascending and descending serotonergic pathways in lampreys and jawed vertebrates.

Keywords: 5-Hydroxytryptamine; Ontogeny; Isthmus; Forebrain; *Petromyzon marinus*; Agnathans

Abbreviations: CC, central canal; Ch, optic chiasm; CRG, caudal rhombencephalic group; DF, dorsal funiculus; DIC, dorsal isthmic commissure; DIS, dorsal isthmic subgroup; DTS, dorsal tuberal subgroup; DTh, dorsal thalamus; DV, diencephalic ventricle; Fr, fasciculus retroflexus; Gl, olfactory glomeruli; H, hypothalamus; Hb, habenula; Hyp, hypophysis; IG, isthmic group; IGL, inner granular layer; IN, interpeduncular nucleus; IR, infundibular recess; LF, lateral funiculus; LP, lateral pallium; M, mesencephalon; M1-3, Müller cells; MI, isthmic Müller cell; MIS, middle (intermediate) isthmic subgroup; Mm, mammillary region; MP, medial pallium; MT, mesencephalic tegmentum; MV, mesencephalic ventricle; OB, olfactory bulbs; OG, olfactory glomeruli; ON, optic nerve; OT, optic tectum; P, pineal organ; PC, posterior commissure; PO, preoptic area; PoC, postoptic commissure; Pp, parapineal organ; PR, posterior recess; Pt, pretectum; PTl, primordium telencephali; RV, rhombencephalic ventricle; SC, spinal cord; ScO, subcommissural organ; SFGS, stratum fibrosum et griseum superficiale; SOP, stratum opticum; Str, striatum; T, tuberal region; TL, telencephalic lobe; T-Mm, tubero-mammillary region; TrG, caudal isthmic subgroup; TrN, trigeminal motor nuclei; TS, torus semicircularis; TV, telencephalic ventricle; VF, ventral funiculus; VIC, ventral isthmic commissure; VIS, ventral isthmic subgroup; VTS, ventral tuberal subgroup; VTh, ventral thalamus; Zli, zona limitans intrathalamica

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1. Introduction

Serotonin is an important neurotransmitter in the brain of vertebrates. The serotonergic system was one of the first components of the central nervous system (CNS) to be characterized histochemically (Parent, 1981, 1984; Parent and Northcutt, 1982). Evolution of the serotonergic system in vertebrates appears to involve progressive disappearance of rostral (forebrain) populations and an increase in the number of brainstem populations (Parent, 1981, 1984; Pierre et al., 1992). Serotonin appears early on in development of the central nervous system (mammals: Frankfurt et al., 1981; Lidov and Molliver, 1982; Wallace and Lauder, 1983; Botchkina and Morin, 1993; chick: Wallace, 1985; anurans: van Mier et al., 1986; Zhao and Debski, 2005; teleosts: Ekström et al., 1985; Bolliet and Ali, 1992; Bolliet et al., 1994; McLean and Fetcho, 2004). In addition to its role as a neurotransmitter that regulates basic types of behaviour, serotonin is involved in the regulation of neuronal development (Lauder and Krebs, 1978; Gromova et al., 1983; Haydon et al., 1984; Chubakov et al., 1986; Lauder, 1987; Goldberg and Kater, 1989; Goldberg et al., 1991; Meier et al., 1991; Lauder, 1993; Gaspar et al., 2003; Sodhi and Sanders-Busch, 2004). Recently, a role for serotonin in the regulation of programmed cell death during brain development has also been suggested (Persico et al., 2003).

Lampreys belong to the most ancient group of extant vertebrates and they have a complex life cycle. In the sea lamprey, the embryonic period (about 12 days) is followed by a non-feeding prolarval period (about 24 days) and a microphagous larval period (several years long), during which the animals are blind and live buried in burrows in the river where they were born. During a complex metamorphosis, larvae transform into young adult lampreys, which, in the European breed, descend to the sea to feed parasitically on fish and then grow rapidly until they finally ascend the river to breed and subsequently die (Hardisty and Potter, 1971).

The serotonergic system in the brain of adult lampreys has been investigated by formaldehyde-induced fluorescence (Honma, 1969; Baumgarten, 1972) and by immunohistochemistry (Steinbusch and Nieuwenhuys, 1979; Steinbusch et al., 1981; Brodin et al., 1986, 1988; Pierre et al., 1992; Viana di Prisco et al., 1994; Antri et al., 2006). The presence of a single group of serotonergic cells has been reported in embryos and early larvae of the river lamprey (Hay-Schmidt, 2000), and there are some reports of the presence of serotonergic cells in larval sea lampreys (Yáñez, 1992; Meléndez-Ferro et al., 2002a,b; Antri et al., 2006). The previous study of Antri et al. (2006) did not examine the early developmental stages of lampreys and all serotonergic populations found in adults were already present in all larval stages studied (110–160 mm in length), which may be considered as premetamorphic stages. Therefore, little is known about the time of first appearance or the spatio-temporal developmental patterns of serotonergic cell populations. Knowledge of the early organization of this system in lampreys may provide important data on the phylogeny of the vertebrate serotonergic system. In the present study, we describe the appearance and development of the different serotonergic populations in the CNS of specimens of sea lamprey ranging from embryos to adults, and also provide information on the early appearance and maturation of the serotonergic circuitry.

2. Materials and methods

2.1. Subjects

Late embryos (E9–E11; $n = 5$), prolarvae (P0–P23; $n = 15$), larvae (9–170 mm in length; $n = 20$), metamorphic individuals ($n = 10$), postmetamorphic ($n = 10$) and sexually mature adults ($n = 5$) of the sea lamprey *Petromyzon marinus* were analysed. Embryos and prolarvae were obtained in the laboratory from *in vitro* fertilized eggs (the eggs and sperm were obtained from sexually mature adult lampreys caught in the river Ulla, Galicia, northwest Spain). Eggs and embryos were maintained under appropriate conditions of darkness, temperature (16 °C) and aeration. Larval, metamorphic and postmetamorphic specimens were caught in the river Ulla, whereas adult individuals were purchased from a local supplier. All specimens were maintained in an aerated aquarium until processing. All experiments were conducted in accordance with European Community guidelines on animal experimentation.

Embryos and prolarvae were staged by age (e.g. E11 indicates 11 days post-fertilization embryos; P4 indicates 4 days post-hatching prolarvae, etc.). The broods hatched 11–12 days after fertilization of the eggs. In order to further classify the prolarvae, the stages defined for the sea lamprey by Piavis (1971) were used: hatching (P0 and P1), pigmentation (P2 and P3), gill cleft (P4–P7), and burrowing (P8–P23). Larvae were classified by total body length and metamorphic individuals of *P. marinus* were staged as M1–M7, according to Youson and Potter (1979).

2.2. Serotonin immunohistochemistry

Before the experiments, the animals were deeply anaesthetized with 0.05% benzocaine (Sigma, St. Louis, MO) in fresh water. All embryos and prolarvae, heads of larvae and brains of adults were fixed by immersion in freshly prepared 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4 (PB) for 4–6 h. Two sectioning methods were used. (A) After rinsing in PB, some samples were cryoprotected with 30% sucrose in PB, embedded in Tissue Tek (Sakura, Torrance, CA), frozen using liquid nitrogen-cooled isopentane, and cut on a cryostat. (B) Other samples were dehydrated in a graded alcohol series, cleared in xylene, embedded in paraffin wax and cut on a rotary microtome. In both cases, transverse and sagittal sections (10–16 μm thickness) were mounted on gelatin-subbed slides.

For immunohistochemistry, all antibodies were diluted in phosphate buffered saline (PBS) containing 3% normal serum and 0.2% Triton X-100 as detergent. Non-specific binding sites in the sections were blocked by incubation with 10% normal goat serum (Vector Laboratories, Burlingame, CA) in PBS. The sections were then incubated overnight in a humid chamber at room temperature, with a rabbit polyclonal anti-serotonin antibody (Incstar, code no. 20080; lot no. 051007; dilution 1:500 for the indirect immunofluorescence method or 1:5000 for the peroxidase–antiperoxidase (PAP) method) raised against serotonin coupled to bovine serum albumin (BSA) with paraformaldehyde. The immune complex was visualized either by the PAP method or the indirect immunofluorescence method. In the first case, the sections were first rinsed in phosphate buffered saline (PBS; pH 7.4) and then incubated with a goat anti-rabbit antibody (Sigma, code no. R3382; diluted 1:100), for 1 h at room temperature. The sections were incubated in peroxidase–antiperoxidase complex (PAP complex, Sigma, code no. P2026; diluted 1:400), rinsed and developed with 0.6 mg/ml 3-3′diaminobenzidine (Sigma) and 0.003% H_2O_2 in PBS. The sections were developed, then rinsed in distilled water, dehydrated, and coverslipped with Eukitt (Panreac, Barcelona, Spain). For indirect immunofluorescence detection of serotonin, a FITC-conjugated swine anti-rabbit antibody (Dako, code no. F0205; diluted 1:30) was used. The sections were rinsed in distilled water, then coverslipped