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Temporal and spatial organization of tyrosine hydroxylase-immunoreactive cell groups in the embryonic brain of an elasmobranch, the lesser-spotted dogfish *Scyliorhinus canicula*

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Abstract

We have studied the development of catecholaminergic (CA) neuronal groups in the brain of the dogfish *Scyliorhinus canicula* using immunohistochemistry to tyrosine hydroxylase (TH). The earliest TH-immunoreactive (THir) cells were detected in the primordia of the posterior tubercle and suprachiasmatic nuclei (PTN and SCN, respectively) of stage 26 embryos. At stage 28, THir cells were also seen extending between the SCN and the PTN at ventral thalamic levels. At stage 30, some THir cerebrospinal fluid-contacting neurons and migrated THir cells were found in the walls of the posterior recess, and a few weakly THir cells also appeared at the isthmus level (locus coeruleus) and in the caudal rhombencephalic tegmentum. At stage 31, further THir cell groups appeared in the synencephalon and midbrain (ventral tegmental area/substantia nigra, VTA/SN), and the rhombencephalon (viscerosensory and visceromotor columns). At stage 32, the first THir cells appeared in the pallium, the olfactory bulb and the preoptic area. THir cells are seen in the retina from stage 33. The developmental sequence of THir cell groups in dogfish appears to be rather similar to that described for teleosts, apart from the appearance of the VTA/SN and pallial cells, which lack in teleosts.

Keywords: Catecholaminergic system; Immunohistochemistry; Cartilaginous fishes; Evolution

1. Introduction

Developmental studies are essential for understanding the functions and evolution of catecholaminergic (CA) systems. Whereas the distribution of catecholaminergic neurons has been thoroughly studied in a number of species covering all major vertebrate groups [14], the development of CA systems has only been studied in the brain of a few species: mammals [5,14], chick [13], a lizard [9], amphibians [6,7], two teleosts [4,8] and lamprey [12].

The brain of the lesser-spotted dogfish *Scyliorhinus canicula* (in the following referred to simply as dogfish) has been the subject of a number of hodological and immunohistochemical studies, and thus can be used as a model for the elasmobranch brain. Elasmobranchs possess well developed CA systems, the organization of which in the adult brain has

Abbreviations: Cb, cerebellum; cc, central canal; cVTA, caudal VTA; Di, diencephalon; fr, fasciculus retroflexus; Ha, habenula; H, hypophysis; IHL, inferior hypothalamic lobe; Lamt, lamina terminalis; LC, locus coeruleus; Mes, mesencephalon; OB, olfactory bulb; OT, optic tectum; P, pallium; Pc, posterior commissure; Pi, pineal gland; PO, preoptic area; Pr, posterior recess; Pro/PRO, posterior recess organ; PTN, posterior tubercle nucleus; R, Rathke's pouch; Ret, rhombencephalic reticular formation; Rh, rhombencephalon; Rpo, preoptic recess; rVTA, rostral VTA; SCN, suprachiasmatic nucleus; Si, saccus infundibuli; SN, substantia nigra; Spc, spinal cord; SuC, subcoeruleus reticular nucleus; SV, saccus vasculosus; Syn, synencephalon; Tel, telencephalon; Tv, ventral thalamus; VMC, visceromotor column; VSC, viscerosensory colum; Vt, velum transversum; VTA, ventral tegmental area

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been studied in several species and shows a rather consistent pattern [15]. In the adult dogfish, however, only the CA systems of the hypothalamus have been studied in detail [10]. Recently, we have also analyzed the development of CA systems in the spinal cord of the dogfish [16]. The aim of the present study was to analyze the sequence of appearance and the distribution of the various CA cell groups in the brain of the developing dogfish, using immunocytochemistry to tyrosine hydroxylase (TH), the initial rate-limiting enzyme of the catecholamine synthesis pathway.

2. Material and methods

The embryos used were kindly provided by the Aquarium "Vasco de Gama" and Oceanarium of Lisbon (Portugal). Stages 25–34 (prehatching), staged according to [3], were used (two to four specimens of each stage). The embryos were separated from the yolk sac and fixed by immersion in 4% paraformaldehyde in elasmobranch phosphate buffer (0.1 M phosphate buffer containing 670 mM urea) at pH 7.4 during 10 h. In addition, four juvenile and four adult dogfish were fixed by vascular perfusion with the same fixative after deep anesthesia in 0.05% tricaine methane sulfonate in seawater. After fixation, whole embryos and brains of juveniles and adults were cryoprotected, frozen, and serially sectioned on a cryostat in transverse or sagittal planes (14–16 μm thickness). Sections were mounted on gelatin-coated slides, and processed immunohistochemically with a monoclonal TH antibody (Chemicon, Temecula, CA; dilution, 1:1000) and the PAP method, as elsewhere [16].

3. Results

The first TH-immunoreactive (THir) neurons of the brain are detected in the diencephalon of stage-26 embryos, i.e. at the same stage in which the first THir cells appear in the spinal cord floor [16] (Table 1). Most of them are found in the caudal hypothalamus/posterior tubercle (Figs. 1a and 2a), although a few THir cells are located in the caudal walls of the posterior recess (Fig. 2a). At this stage, a few weak THir cells are also seen in the region of the postoptic commissures (Fig. 1a). These diencephalic THir populations form the primordia of the posterior tubercle nucleus (PTN), the posterior recess organ (PRO) and the suprachiasmatic nucleus (SCN), respectively. In stage-28 embryos, a continuous band of THir cells extends between the SCN and the PTN at ventral thalamic levels. Moreover, in the PRO some THir cells show a ventricular process (CSF-contacting cells), while others are located superficially and extend processes parallel to the outer surface (Fig. 2b). At stage-30 embryos, the number of diencephalic THir cells and fibres in the PTN and SCN has increased notably with respect to previous stages (Figs. 1b, 2c and d). In contrast, the number of THir cells in the PRO does not increase appreciably with development (Fig. 2d). In addition, a few weak THir cells appear in subventricular position in the isthmus representing the locus coeruleus primordium (Table 1; Fig. 1b), and a few THir cells are seen in the caudal rhombencephalic tegmentum, representing catecholaminergic cells of the caudal reticular formation (Table 1; Figs. 1b and 2e). At stage 31, two clearly separated groups of weakly stained THir cells, rostral and caudal, can be recognized in the synencephalic and mesencephalic tegmentum: the rostral group is located around the basis of the fasciculus retroflexus (synencephalon), whereas the caudal group is located at the level of the III nerve (midbrain). The rostral group gives rise to conspicuous medial and lateral THir populations, the rostral ventral tegmental area (VTA) and the substantia nigra (SN), respectively. The midbrain group only originates faintly stained cells located medially at the level of the oculomotor roots (caudal VTA). At this stage, the isthmus shows two separated THir populations, rostromedial (locus coeruleus) and caudolateral (subcoeruleus reticular nucleus). THir cells are also found in the caudal rhombencephalon,

Table 1
Sequence of appearance of THir cell groups in the dogfish brain during development