

COMPARATIVE STUDY OF THE CITOARCHITECTURE AND NEUROCHEMICAL CONTENT OF *AREA POSTREMA* IN THREE SPECIES OF FISH: *SALMO TRUTTA FARIO* L., *ACIPENSER BAERI* BRANDT AND *SCYLLIORHINUS CANICULA* L.

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Abstract. *Area postrema* (AP) is a neurohemal organ located at the transition between rhombencephalon and spinal cord, whose structure and functions have been well studied in mammals but poorly known in fishes. In the present study we characterize the structure of AP in three species of fish, the brown trout (*Salmo trutta fario* L.), the Siberian sturgeon (*Acipenser baeri* Brandt) and the dogfish (*Scyliorhinus canicula* L.). Comparing the results obtained by using immunohistochemical techniques, we identified similarities, but also differences between the cytochemical structures of the AP in these three species. The cytoarchitecture is basically similar, all having the same stratified organization. The differences appeared in the neurochemical content of the neurons in this zone. It seems like evolution kept the basic structure but modified the neurotransmitters used by the cells and the fibers system.

Keywords: *Area postrema*, Immunohistochemistry, *Scyliorhinus canicula*, *Acipenser baeri*, *Salmo trutta fario*, Evolution.

Rezumat. Studiul comparativ al citoarhitecturii și conținutului neurochimic al ariei *postrema* la trei specii de pești: *Salmo trutta fario* L., *Acipenser baeri* Brandt și *Scyliorhinus canicula* L. *Area postrema* (AP) este un organ neurohemal localizat la trecerea dintre rombencefal și măduva spinării. Structura și funcțiile sale au fost bine studiate la mamifere, dar sunt puțin cunoscute la pești. În prezentul studiu este caracterizată structura zonei AP la trei specii de pești, *Scyliorhinus canicula*, *Acipenser baeri* și *Salmo trutta fario*. Comparând rezultatele obținute prin tehnici imunohistochimice, au fost identificate similități, dar și diferențe între structurile citochimice ale zonei AP la cele trei specii. Citoarhitectura este în general similară, la toate speciile existând o organizare stratificată. Diferențele au apărut în conținutul chimic al neuronilor din AP. Se pare că evoluția a păstrat structura de bază, dar a modificat neurotransmițătorii folosiți de celulele și sistemul de fibre.

Cuvinte cheie: *Area postrema*, imunohistochimie, *Scyliorhinus canicula*, *Acipenser baeri*, *Salmo trutta fario*, evoluție.

Introduction

Circumventricular organs are described as parts of the ventricular wall, situated in the midsagittal plane or, like the choroid plexus, developed thereof. The composition of their tissue elements differs from the remaining brain. The circumventricular organs (CVOs) occupy thin parts of the ventricular wall and thus not only border on the inner (ventricular) cerebrospinal fluid but also on the outer cerebrospinal fluid (Krisch & Leonhardt, 1989). The area postrema (AP) is the most caudal of the CVOs, being situated at the dorsal surface of the medulla at the obex, just in the place where the fourth ventricle closes. In this particular part of the brain there is no blood-brain barrier, so the neurons are contacting directly with some chemical compounds of the blood that normally are too big to cross the blood-brain barrier (Morita & Finger, 1987).

The AP is organized in a unique laminar fashion, from superficial to deep: meninx, vasculature, palisade layer, layer of neuronal somata, and layer of ventral neuropil. This structure is common to all three species.

The exact function of the AP is not certain although this area may serve as direct chemoreceptive interceptors for detection of some blood-borne compounds, but also receives inputs from other visceral afferent systems. Various investigators have proposed diverse functions for the AP in mammals, functions like: vomiting and nausea control, cardiovascular regulation (by activating the nicotinic ACh receptors), feeding and drinking behaviour and body fluid homeostasis. For example, the AQP4-aquaporins (cell membrane and water channels protein) discovered in AP in rats and chickens where they play a crucial role in volume homeostasis in the body including several pathological conditions such as formation of brain edema (Butler & Hodos, 2005). In human case, the microvasculature of the AP region also plays a role in connecting AP and STN (nucleus tractus solitarius, an important regulatory centre of cardiovascular and respiratory functions), between them being identified a portal system, by means of which postremal venous drainage conveys neuroactive factors to STN (Porzionato *et al.*, 2005). Receptor binding studies indicate that the AP contains neurons that may be responsive to opiates, dopamine, nor epinephrine, angiotensin II, insulin and cholecystokinin (Morita and Finger, 1987).

The *area postrema* (AP) had been believed to occur only in mammals (as cats, dogs, rats), birds (as pigeons), and amphibians (as frogs and salamanders) (Morita & Finger, 1987). Recently though, the AP has been identified in various fishes, cartilaginous (sharks) and bony fishes (Morita & Finger, 1987).

Apparently, the cells of this region use for communication a great variety of neurotransmitters, the most common being the catecholamines, identified by their rate-limiting enzyme, namely tyrosine hydroxylase.

Material and Methods

For the study, adult specimens of brown trout (*Salmo trutta fario* L.), Siberian sturgeon (*Acipenser baeri* Brandt) and dogfish (*Scylliorhinus canicula* L.) were used. A commercial supplier from Spain provided them. All animals were deeply anaesthetised with tricaine methane sulfonate (MS-222, Sigma, St. Louis, MO) at a concentration of 0.5 g/l in salt or sweet water (depending on the life medium of the fish). The specimens were then perfused through the conus/bulbus arteriosus with Ringer's solution containing 0.1% procaine (Sigma), followed by cold 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4 (PB). The brain and spinal cord were removed and left in the same fixator for 24 hours at 4° C. After fixation, the brains were crioprotected with 30% sucrose, embedded in OCT compound (Tissue Tek, Torrence, CA) and frozen with liquid nitrogen cooled isopentane. The embedded tissue was then cutted (14-16 µ), transversally and sagittally, on a cryostat and the sections were then mounted on Super Frost slides. We also used paraffin embedded brains, which were sectioned at 14-16 µ on a microtom and mounted on chrome alum-gelatinised slides. All procedures were conformed to the European Community Guidelines on Animal Care and Experimentation.

The material was processed using the peroxidase-antiperoxidase method (PAP) and the immunofluorescence method, with monoclonal and polyclonal antibodies against TH (tyrosine hydroxylase), 5-HT (serotonin), GAL (galanin), SOM (somatostatin), NPY (neuropeptide Y) and CGRP (calcitonin gene related peptide). The paraffin embedded sections were processed separate from those of cryostat. For paraffin embedded tissue: deparaffination in xylene (3 baths of 20 minutes each), rehydration in ethanol (100°, 96°, 70° for 20 minutes each), washing in buffer (PBS, TBS-T, TrisCl), antigen retrieval at 90°C in Citrat buffer in water bath, 30% H₂O₂ for 30 minutes, washing in buffer, the first antibody (at the concentration recommended by the provider for each of them) over night in the humid chamber, washing in buffer, second antibody (at the concentration recommended by the provider for each of them) for 1 hour in the humid chamber, washing

in buffer, PAP complex. The immunoreaction was revealed with 0.05% diaminobenzidine (DAB) in 0.003% H₂O₂, simple (brown) or with Ni (blue). The cryostat sections were rinsed in PBS (3x10 minutes) and the rest of the procedure beginning with the adding of the first antibody was identical.

For double immunofluorescence we mixed the 2 first antibodies and incubated them over night in a humid chamber, then rinsing in buffer, the mix of the 2 secondary antibodies with 2 different fluorochromes for 1 hour in a dark place, rinsing in buffer and then in distilled water and mounted in VECTASIN.

We also used the Haematoxylin-Eosin method for identifying the general structure. The slides were rinsed in Haematoxylin for 10 minutes and washed in water, the rinsed in Alcoholic Eosin for 1 minute, and then dehydrated in series of ethanol and clarified in xylene. All slides were mounted in Eukit.

The results were then observed at both optic and confocal microscope.

Results

a. AP anatomy

In trout, the AP is situated outside of the brain fourth ventricle, so the dorsal part of AP is exposed to the intracranial fluid; the AP appears just where the *corpus cerebelli* ends (dorsal view), where the fourth ventricle closes (sagittal view). Near to this dorsal neurohemal organ, there can be identified the roots of the vagus nerve, and the *nucleus comisurali* of Cajal, and just below is the fourth ventricle. According to staining by Haematoxylin-Alcoholic Eosin method, the AP of trout has a laminar structure (Fig. 1). The first zone (zone I) seems to be the meninx. Like in other organisms, in certain regions of the central nervous system (CNS), neural tissue is absent, but the meninx persists. The second zone (zone II) of the AP is represented by the blood vessels (sinus capillaries) and the connective tissue in a broad perivascular space. In a transversal section, the blood vessels seem to be arranged in a row. Of course, the capillaries of this zone are known to be fenestrated, a typical thing for the CNS regions lacking the blood brain barrier (Morita & Finger, 1987). The third zone (zone III) has to be the palisade layer, but in trout from our observations, we cannot tell for sure that this layer exists. In literature, dendritic processes of AP neurons and astrocytes are described in this layer (Morita & Finger, 1987). The dendrites are reaching towards the external basal lamina of the capillaries. The astrocytes have a particular form in transverse section, a shape of elaborate candelabra. The fourth zone (zone IV, layer of neuronal somata) is composed by the perikarya and ramifications of AP neurons and astrocytes. The fifth zone (zone V) is the layer of the basal dendrites of AP neurons that extend ventrally and that are distributed in the commissural nucleus of Cajal.

Though not so well delineated as in trout, the main layered structure of the AP can be observed also in sturgeon and dogfish.

b. The neurochemical content of the AP perikarya and fibres system

***Salmo trutta fario* L. (brown trout)**

A strong immunoreactivity to TH was observed at the AP level. As signals the literature, at this level, the transition zone between rhombencephalon and medulla, there is one of the most obvious groups of neurons that possess this enzyme. This large group contains medium and big size neurons, in an obvious relation with the meninx and the blood vessels by short and very ramified dendrites. A high density of TH positive fibres can also be observed, fibres that come from other levels or go to other nuclei. This strong immunoreactivity to TH has been observed all along the AP, from rostral to caudal, decreasing towards the medulla. Serotonin is another well spread neurotransmitter in vertebrates' brain. At the AP level, only fibres with serotonin had been observed, fibres

that are only on the lateral sides, just crossing by (Fig. 2.a). Galanin has been identified in perycarya and fibres. The GAL positive cells are in a small number, but the fibre system is quite well developed, some of them contacting with the blood vessels from above (Fig. 3.a). The Neuropeptide Y is present only in fibres at this level (Fig. 4.a). Calcitonin Gene Related Peptide appears only in some fibres at the AP level, crossing from one side to the other, very well observed in a sagittal section (Fig. 5.a). Somatostatin was also identified at this level, in fibres that use this area as a bridge towards other levels.

Acipenser baeri Brandt (Siberian sturgeons)

The TH positive fibres and perycarya are also present in sturgeon at the AP level, though the density of them is smaller than in trout. The general distribution is similar to that in trout (Fig. 2.c). Serotonin was observed marking fibres of the AP, in a decreased number and only on the lateral sides, not reaching the blood vessels (Fig. 2.d). Galanin was identified only in fibres at the AP level in sturgeon (Fig. 3.b), but the NPY distribution is similar to that of TH in trout (Fig. 4.b). The NPY has been identified in fibres and neurons, appearing as a co-localization of the two neurotransmitters in some areas. The results for CGRP were not so conclusive due to the malfunctioning of the antibody or probably to the fact that this neurotransmitter is not so used at this level in sturgeon's AP. No cells with somatostatin were identified, only some fibres on the lateral sides of this area.

Scylliorhinus canicula L (dogfish)

The catecholaminergic system seems to be less developed at the AP level in dogfish than in the other two species. Still, TH is present in both cells and fibres (Fig. 2.b). Serotonin is present in fibres at the AP level, fibres quite intense positive that seems to reach the blood vessels. No serotonin positive cells were identified. The immunoreactivity to galanin was very weak, represented by some fibres and isolated cells at the inner face of the fourth ventricle (Fig. 3.c). As in sturgeon, the immunoreactivity to NPY was very high, in both cells and fibres, some of them reaching the sinus capillaries (Fig. 4.c). As for CGRP, it appeared alternative with the TH system. So CGRP appeared more rostral in cells and fibres, then together with TH, which was more abundant near the ventricle, and CGRP at the exterior face, dorsally, and only in fibres (Fig. 5.b). Somatostatin was identified only in fibres, in an increased number than in the other two species, probably reaching the blood vessels (Fig. 5.c).

Discussion

The cytoarchitecture of the *area postrema* seems to be a conserved structure in evolution. Though the structure of the AP in dogfish is more diffuse, this neurohemal organ surely exists in this specie. The Siberian sturgeon has an intermediary structure, and the AP in trout is more similar to that of mammals. We cannot speak though about the same functions of the AP in fish as in humans where there is an important control centre of emesis for example. This structure appeared early in animals life and gain more and more importance while climbing the stairs of evolution.

At this "primitive" level of AP's development naming these aquatic animals, fish, the differences though not so well defined appeared concerning the dimensions, the rostro-caudal extension and the neurochemical content.

The catecholaminergic system of the AP appeared early in evolution. Though the cyclostomes do not have this neurohemal organ, and the group of cathecoaminergic neurons are situated only at the ventral part of the obex (Pierre *et al.*, 1997), the elasmobranches begin to have it, not so well developed but still. In chondrostei (sturgeons), the literature confirms our results about the abundance of the system (Adrio *et*

al., 2002). In teleosts the literature confirms our data for: *Carassius auratus* (Morita & Finger, 1987; Hornby *et al.*, 1987), *Anguilla anguilla* (Roberts *et al.*, 1989), *Gasterosteus aculeatus* (Ekstrom *et al.*, 1992), *Apteronotus leptorhynchus* (Sas *et al.*, 1998), *Gnathonemus petersii* (Meek & Joosten, 1993), *Solea senegalensis* (Rodriguez-Gomez *et al.*, 2000), *Tinca tinca* (Brinon *et al.*, 1998). The AP of amphibians seems to possess large similarities with that of mammals (Franzoni *et al.*, 1986). In mammals, some studies reveal the great distribution of catecholamines at the AP level in cats (Reiner & Vincent, 1986), rodents (Vincent, 1988), and primates (Weihe *et al.*, 2006). We may conclude that the catecholaminergic system evolved with the development of some functions of the AP, more and more complex and more connected with other nervous centres.

The prolongations of serotonergic neurons are wide distributed in the nervous system, including the AP zone. Our data, compared with those from literature confirmed us that this is common for other groups of vertebrates too: amphibians (Ueda *et al.*, 1984; Fasolo *et al.*, 1986), reptiles (Bennis *et al.*, 1990), and mammals (Funahashi *et al.*, 2004; Minami *et al.*, 1996).

If in *Lampetra fluviatilis* (Jimenez *et al.*, 1996) and in dogfish, at the AP level, the galaninergic fibres are rare, and the galaninergic neurons are missing, in the other groups of vertebrates seems that this peptide has a greatest importance in neurocommunication; more, in some fishes researchers discovered a sexual dimorphism concerning the distribution of GAL within the brain (Rodriguez *et al.*, 2003). So at the AP level, not only in chondrosteans, but also in teleosts and other vertebrates like: amphibians (Lazar *et al.*, 1991), reptiles (Jimenez *et al.*, 1994), birds (Josza & Mess, 1993) and mammals - rats (Melander *et al.*, 1986) and primates (Kordower & Mufson, 1992), the net of fibres that use GAL as neurotransmitter is very well developed. In primates, the wide distribution of the galaninergic system in the central nervous system may modulate a lot of cognitive, sensorial and motor processes (Kordower & Mufson, 1992).

The net of somatostatin fibres is quite well developed at the level of medulla oblongata, though we could not find some certain information about the direct contact with the blood vessels. Somatostatin is wide spread in the CNS, but data about the existence of this peptide at the exact level of AP were not found: (Vallarano *et al.*, 1997; Mathieu *et al.*, 2004; Weindl *et al.*, 1984; Laquerriere *et al.*, 1989; Madeo *et al.*, 2005).

If in dogfish and sturgeon the neuropeptidergic system is very well developed at the AP level, in cells and fibres, in teleosts this system is represented only by NPY positive fibres, in omega shape, that seem to close the fourth ventricle dorso-lateral (Castro *et al.*, 1999). This neuropeptide is in a close relation with the structures from the central level implicated in the feeding process (Kuenzel & McMurtry, 1988).

CGRP appears marking cells and fibres at the rostral part of the AP in elasmobranchs, and in teleosts appears only in fibres that cross in this zone, without an apparent connection with the blood vessels. In other groups of vertebrates the presence of CGRP is mentioned only in the nuclei from nearby: *tractus solitarius* and the vagal complex (in rats - Skofitsch & Jacobowitz, 1985; Inagaki *et al.*, 1986). Comparison of CGRP results in elasmobranchs and teleosts with those reported in frog and mammals suggests that this peptide may have similar modulator functions in these vertebrates and that the general pattern of distribution of CGRP in the central nervous system originated in common ancestors that lived more than 200 million years ago (Molist *et al.*, 1995).

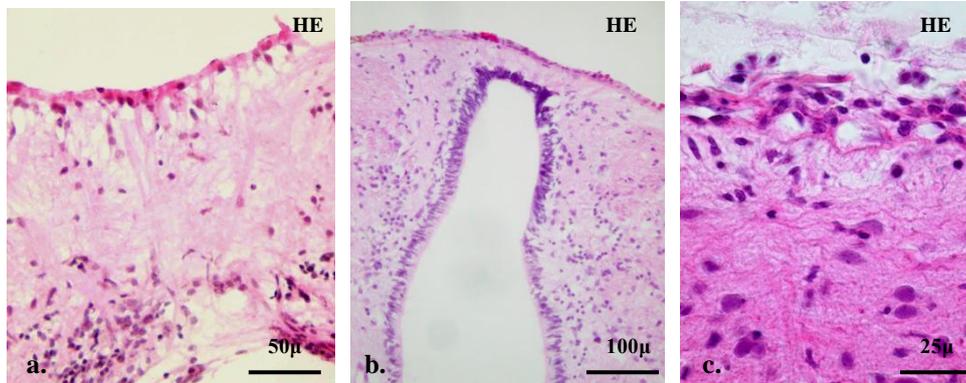


Figure 1. Transversal section through the *Area postrema* (Haematoxylin-Eosin coloration): a. *Salmo trutta fario*; b. *Acipenser baeri*; c. *Scyliorhinus canicula*.

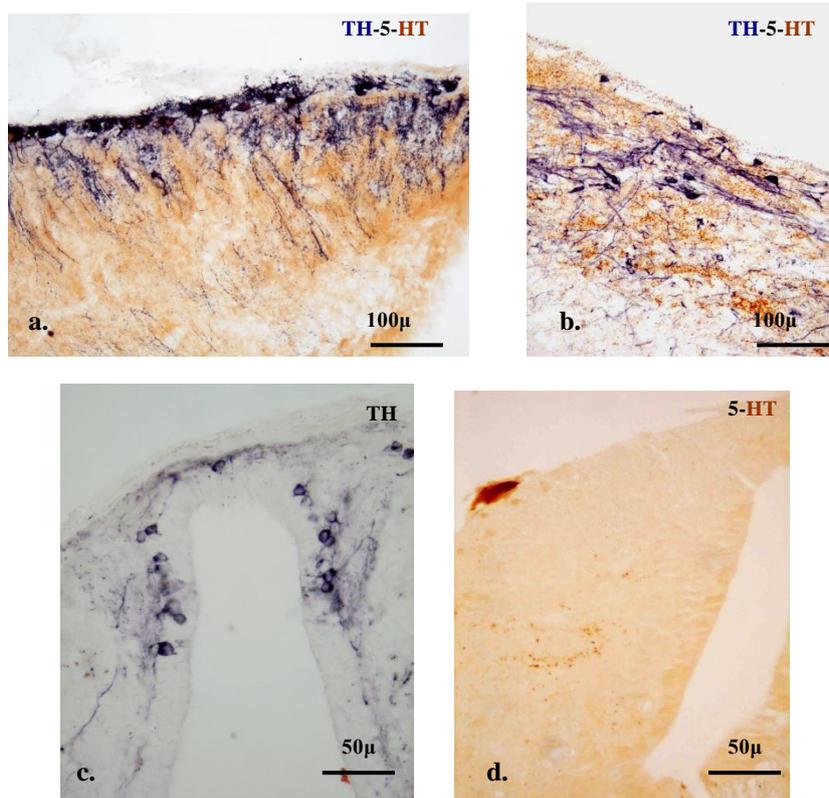


Figure 2. Immunoreactivity to TH and 5-HT: a. *Salmo trutta fario* (sagittal section); b. *Scyliorhinus canicula* (transversal section); c. and d. *Acipenser baeri* (transversal sections).

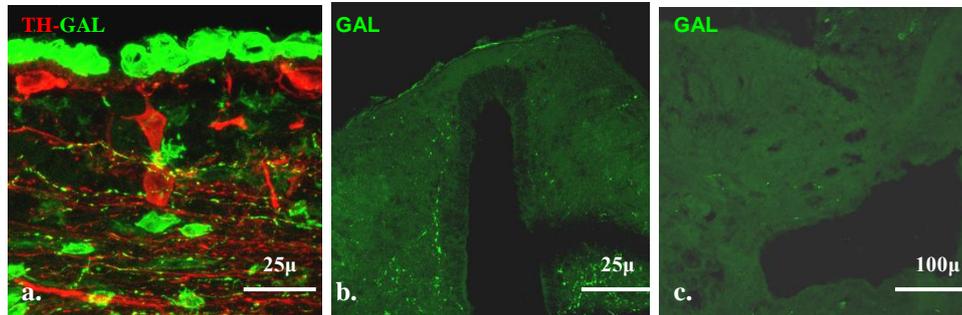


Figure 3. Fluorescent immunoreactivity to TH and GAL (transversal sections): a. *Salmo trutta fario*; b. *Acipenser baeri*; c. *Scyliorhinus canicula*.

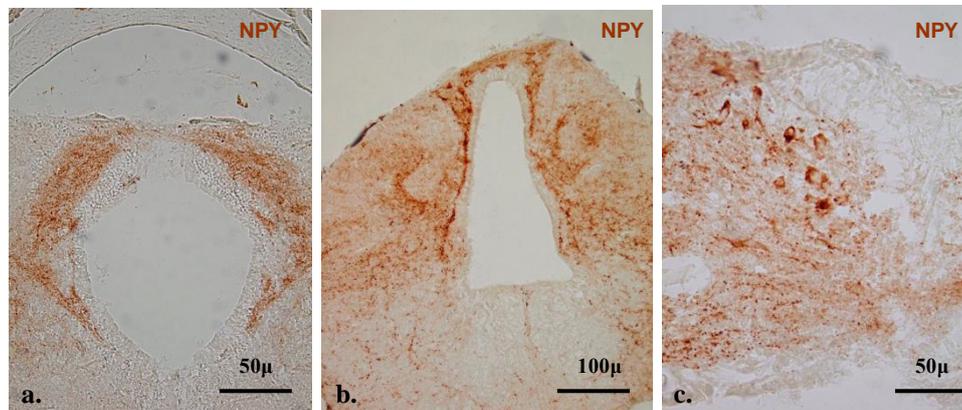


Figure 4. Immunoreactivity to NPY (transversal sections): a. *Salmo trutta fario*; b. *Acipenser baeri*; c. *Scyliorhinus canicula*.

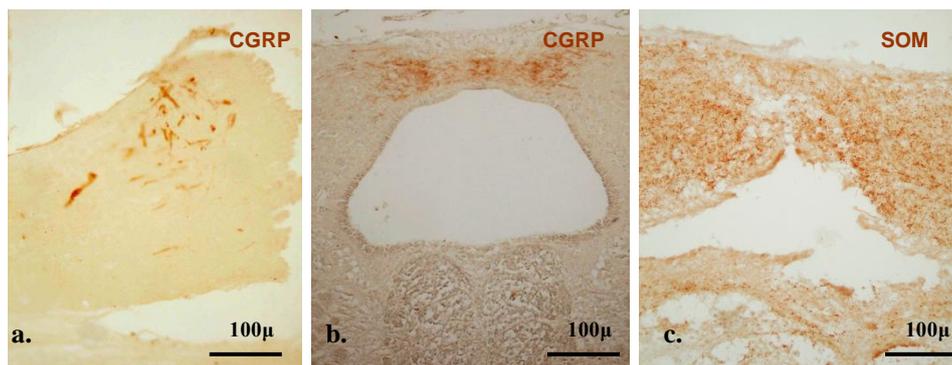


Figure 5. Immunoreactivity to CGRP and SOM: a. *Salmo trutta fario* (sagittal section); b. and c. *Scyliorhinus canicula* (transversal sections).

Conclusions

We may conclude that this neurohemal organ has a general cytoarchitecture similar in all vertebrates, but because of the differences between various mediums of life and the need to adapt to organisms necessities, for the perfect union and integration in it, the structure has been modified by changing the neurochemical content so that the answers could be more exact and rapid.

We have identified a strong reactivity for the enzyme tyrosine hydroxylase (TH), in cells and fibers of the AP in all three species, though their density was higher in both bony fish (especially in trout), than in the elasmobranch. Serotonin, another neurotransmitter widely distributed in the vertebrate's brain, was revealed in abundant serotonergic fibers of the elasmobranch's AP, being poorly distributed in sturgeon and trout. Galanin-immunoreactive cells and fibers were present in the AP of trout, while the galanin system seems to be less developed in sturgeon and dogfish. The Neuropeptide Y (NPY) was also present in the AP of the three species, being especially abundant in dogfish where the distribution of NPY-immunoreactive (ir) cells and fibers reminds that of the TH in trout; in sturgeon this system of NPY-ir cells and fibres is also well developed, while in trout NPY-immunoreactivity was only observed in fibers. Calcitonin gene related peptide (CGRP) was also present in all three species: in dogfish we identified CGRP-ir cells and fibers while in trout CGRP-ir fibers were crossing just in the AP zone; in sturgeon this peptidergic system seems to be poorly developed. We identified fibers containing somatostatin in the lateral parts of the AP, not reaching the blood vessels.

References

- Adrio, F., Anadon, R., Rodriguey-Moldes, I., 2002. Distribution of Tyrosine Hydroxylase (TH) and Dopamine β -Hydroxylase (DBH) Immunoreactivity in the Central Nervous System of two chondrosteian species (*Acipenser baeri* and *Huso huso*). *The Journal of Comparative Neurology*, **448**: 280-297.
- Bennis, M., Gamrani, H., Geffard, M., Calsa, A., Kah, O., 1990. The distribution of 5-HT immunoreactive system in the brain of a saurian, the chameleon. *Journal Hirforsch*, **31** (5): 563-574.
- Brinon, J., Arevalo, R., Weruaga, E., Crespo, C., Alonso, J., Aijon, J., 1998. Tyrosine hydroxylase-like immunoreactivity in the brain of the teleost fish *Tinca tinca*. *Archives Italiennes de Biologie*, **136** (1): 17-44.
- Butler, A.B., Hodos, W., 2005. *Comparative Vertebrate Neuroanatomy-Evolution and Adaptation*, Wiley-Liss, Inc., New York, SUA.
- Castro, A., Becerra, M., Manso, M.J., Anadon, R., 1999. Development of immunoreactivity to Neuropeptide Y in the brain of brown trout (*Salmo trutta fario*). *The Journal of Comparative Neurology*, **414**: 13-32.
- Ekstrom, P., Honkanen, T., Borg, B., 1992. Development of tyrosine hydroxylase, dopamine and dopamine beta hydroxylase immunoreactive neurons in a teleost, the three spined stickleback. *The Journal of Chemical Neuroanatomy*, **5**(6): 481-501.
- Fasolo, A., Franzoni, M.F., Gaudino, G., Steinbusch, H.W., 1986. The organization of serotonin immunoreactive neuronal system in the brain of the crested newt, *Triturus cristatus carnifex* Laur. *Cell Tissue Research*, **243** (2): 239-247.
- Franzoni, M., Thibault, J., Fasolo, A., Martinoli, M., Scaranari, F., Calasa, A., 1986. Organization of tyrosine hydroxylase immunopositive neurons in the brain of the crested newt, *Triturus cristatus carnifex*. *Journal of Comparative Neurology*, **251** (1): 121-134.
- Funahashi, M., Mitoh, Y., Matsuo, R., 2004. Activation of presynaptic 5-HT₃ receptors facilitates glutamatergic synaptic inputs to area postrema neurons in rat brain slices. *Methods and Findings in Experimental and Clinical Pharmacology*, **26** (8): 615-622.
- Hornby, P.J., Piekut, D.T., Demski, L.S., 1987. Localization of immunoreactive tyrosine hydroxylase in the goldfish brain. *Journal of Comparative Neurology*, **261**(1): 1-14.
- Inagaki, S., Kito, S., Kubota, Y., Girgis, S., Hilvard, C.J., MacIntyre, I., 1986. Autoradiographic localization of calcitonin gene-related peptide binding sites in human and rat brains. *Brain Research*, **374**: 287-298.
- Jimenez, A.J., Mancera, J.M., Perez-Figares, J.M., Fernandez-Llebrez, P., 1994. Distribution of galanin-like immunoreactivity in the brain of the turtle *Mauremys caspica*. *Journal of Comparative Neurology*, **349** (1): 73-84.

- Jimenez, A.J., Mancera, J.M., Pombal, M., Perez-Figarez, J.M., Fernandez-Lebrez, P., 1996. Distribution of galanin – like immunoreactive elements in the brain of the adult lamprey *Lampetra fluviatilis*. *The Journal of Comparative Neurology*, **368** (2): 185-197.
- Jozsa, R., Mess, B., 1993. Galanin-like immunoreactivity in the chicken brain. In *Cell Tissue Research*, **273** (2): 391-399.
- Kordower, J., Mufson, E., 1992. Galanin immunoreactivity in the primate nervous system. *The Journal of Comparative Neurology*, **319** (4): 479-500.
- Krisch, B., Leonhardt, H., 1989. Relations between leptomeningeal compartments and the neurohemal regions of circumventricular organs. *Biomedical research*, **10**, supplement 3: 155-168.
- Kuenzel, W., McMurtry, J., 1988. Neuropeptide Y: brain localization and central effects on plasma insulin levels in chicks. *Physiological Behaviour*, **44** (4-5): 669-678.
- Laquerriere, A., Leroux, P., Gonzalez, B., Bodenat, C., Benoit, R., Vaudry, H., 1989. Distribution of somatostatin receptors in the brain of the frog *Rana ridibunda*: correlation with the localization of somatostatin containing neurons. *The Journal of Comparative Neurology*, **280** (3): 451-467.
- Lazar, G., Lipositz, Z., Toth, P., Trasti, S., Maderdrut, J., Merchenthaler, I., 1991. Distribution of galanin-like immunoreactivity in the brain of *Rana esculenta* and *Xenopus laevis*. In *The Journal of Comparative Neurology*, **310** (1): 45-67.
- Madeo, M., Giusi, G., Alo, R., Facciolo, R.M., Carelli, A., Canonaco, M., 2005. Different somatostatin receptor subtypes are operating in the brain of the teleost fish, *Coris julis*. *The Journal of Experimental Zoology*, **303A**: 406-413.
- Mathieu, M., Bruzzone, F., Chartrel, N., Serra, G., Spiga, S., Vallarino, M., Vaudry, H., 2004. Somatostatin in the brain of the cave salamander, *Hydromantes genei* (Amphibia, Plethodontidae): immunohistochemical localisation and biochemical characterisation. *The Journal of Comparative Neurology*, **475** (2): 163-176.
- Meek, J., Joosten H.W., 1993. Tyrosine hydroxylase-immunoreactive cell groups in the brain of the teleost fish *Gnathonemus petersii*. *Journal of Chemical Neuroanatomy*, **6** (6): 431-46.
- Melander, T., Hokfelt, T., Rokaeus, A., 1986. Distribution of galanin-like immunoreactivity in the rat nervous system. *The Journal of Comparative Neurology*, **248** (4): 475-517.
- Minami, M., Endo, T., Hirafuji, M., 1996. Role of serotonin in emesis. *Nippon Yakurigaku Zasshi*, **108** (5): 233-242.
- Molist, P., Rodriguez-Moldes, I., Batten, T., Anadon, R., 1995. Distribution of Calcitonin Gene Related Peptide immunoreactivity in the brain of the small spotted dogfish, *Scyliorhinus canicula* L.. *The Journal of Comparative Neurology*, **352**: 335-350.
- Morita, Y., Finger, Th.E., 1987. Area Postrema of the goldfish, *Carassius auratus*: ultrastructure, fiber connections and immunocytochemistry. *The Journal of Comparative Neurology*, **256**: 104-116.
- Pierre, J., Manouche, M., Suderevskaya, E.I., Reperant, J., Ward, R., 1997. Immunocytochemical localization of dopamine and its synthetic enzymes in the central nervous system of the lamprey *Lampetra fluviatilis*. *The Journal of Comparative Neurology*, **380**: 119-135.
- Porzionato, A., Macchi, V., Morsut, L., Parenti, A., De Caro, R., 2005. Microvascular patterns in human medullary tegmentum at the level of the area postrema. *Journal of Anatomy*, **206**: 405-410.
- Reiner, P.B., Vincent, S.R., 1986. The distribution of tyrosine hydroxylase, dopamine-beta-hydroxylase, and phenylethanolamine-N-methyltransferase immunoreactive neurons in the feline medulla oblongata. *Journal of Comparative Neurology*, **248** (4): 518-531.
- Roberts, B.L., Meredith, G.E., Maslam, S., 1989. Immunocytochemical analysis of the dopamine system in the brain and spinal cord of the European eel, *Anguilla anguilla*. *Anatomy and Embryology (Berl)*, **180** (4): 401-412.
- Rodriguez, M.A., Anadon, R., Rodriguez-Moldes, I., 2003. Development of Galanin-Like immunoreactivity in the brain of the brown trout (*Salmo trutta fario*), with some observations on sexual dimorphism. *The Journal of Comparative Neurology*, **465**: 263-285.
- Rodriguez-Gomez, F.J., Rendon-Unceta, M.C., Sarasquete, C., Munoz-Cueto, J.A., 2000. Localization of galanin-like immunoreactivity structures in the brain of the senegalense sole, *Solea senegalensis*. *Journal of Histochemistry*, **32** (2): 123-131.
- Sas, E., Maler, L., Tinner, B., 1998. Catecholaminergic systems in the brain of a gymnotiform teleost fish: an immunohistochemical study. *The Journal of Comparative Neurology*, **292** (1): 127-162.
- Skofitsch, G., Jacobowitz, D., 1985. Immunohistochemical mapping of galanin-like neurons in the rat nervous system. *Peptides*, **6** (3): 509-546.
- Ueda, S., Nojvo, Y., Sano, Y., 1984. Immunohistochemical demonstration of the serotonin neuron system in the central nervous system of the bullfrog, *Rana catesbeiana*. *Anatomy and Embryology (Berl)*, **169**: 219-229.
- Vallarino, M., Trabucchi, M., Masini, M., Chartrel, N., Vaudry, H., 1997. Immunocytochemical localization of somatostatin and autoradiographic distribution of somatostatin binding sites in the brain of the african lungfish, *Protopterus annectens*. *The Journal of Comparative Neurology*, **388** (3): 337-353.

- Vincent, S.R., 1988. Distributions of tyrosine hydroxylase, dopamine-beta-hydroxylase and phenylethanolamine-N-methyltransferase immunoreactive neurons in the brain of the hamster (*Mesocricetus auratus*). *Journal of Comparative Neurology*, **268** (4): 584-599.
- Weihe, E., Schutz, B., Schafer, M., Eiden, L., 2006. Three Types of Tyrosine Hydroxylase Positive CNS Neurons Distinguished by Dopa Decarboxylase and VMAT2 Co-Expression. *Cellular and Molecular Neurobiology*, 26 (4-6): 659-678.
- Weindl, A., Triepel, J., Kuchling, G., 1984. Somatostatin in the brain of the turtle *Testudo hermanni* Gmelin. An immunohistochemical mapping study. *Peptides* **5** (supliment 1): 91-100.