

Immunocytochemical localization of vasotocin-like immunoreactivity in the brain of the cartilaginous fish, *Scyliorhinus caniculus*

Mauro Vallarino¹, Carla Viglietti-Panzica², and Gian Carlo Panzica²

¹ Istituto di Anatomia Comparata dell'Università di Genova, Genova, Italy;

² Dipartimento di Anatomia e Fisiologia Umana dell'Università di Torino, Torino, Italy

Accepted June 21, 1990

Summary. The distribution of vasotocin-like peptides in the central nervous system of the cartilaginous fish *Scyliorhinus canicula* was determined by indirect immunofluorescence and peroxidase anti-peroxidase techniques, using a specific antiserum raised in rabbits against synthetic vasotocin. Immunoreactive perikarya were mainly detected in the anterior hypothalamus, within the mid-caudal part of the preoptic nucleus. The most rostral positive cell bodies were located in the dorso-lateral parts of the preoptic area, whereas at a more caudal level, they took a ventro-medial position within the deepest layers of the nucleus. Throughout the preoptic region these cells varied in shape according to their location. Occasionally, scattered vasotocin-like immunopositive cells were also identified in the nucleus periventricularis hypothalami. Vasotocin immunoreactivity was detected in numerous varicose nerve fibers of the preoptico-hypophysial tract. These fibers were seen to course through the medio-basal hypothalamus and caudally, after having passed the hypophysial stem, they reached the neurointermediate lobe of the pituitary. Numerous immunoreactive fibers were also observed within the rostro-medial region of the median eminence. At this level the fibers were in close proximity to the capillary loops. In the preoptic region, some stained cells exhibited short processes that appeared to contact non-reactive perikarya. By comparing the distribution of vasotocin- and corticotropin-releasing factor immunoreactivity on adjacent thin serial sections, it was revealed that these peptides, in *S. canicula*, do not coexist in the same perikarya. The present results, are compared with those obtained in other vertebrate groups, and their possible functional implications are discussed.

Key words: Immunocytochemistry – Vasotocin – Hypothalamus – Neurosecretory fibers – *Scyliorhinus canicula* (Elasmobranchii)

Send offprint requests to: Dr. Mauro Vallarino, Istituto di Anatomia Comparata, Università di Genova, Viale Benedetto XV, 5, I-16132 Genova, Italy

The neurohypophysial hormones are particularly useful evolutionary tracers. They are all based on a small molecule composed of 9 amino acids, and substitutions of one or more of these amino acids result in at least 10 different hormones that have been found in the different classes of vertebrates (Acher 1985). According to the hormones discovered in mammals, the different neurohypophysial hormones can be clustered in vasopressin-like and oxtocin-like peptides.

Within the family of vasopressin-like peptides, vasotocin has been found in all vertebrate groups except in adult mammals (Acher 1985).

Several studies have detailed the immunocytochemical distribution of vasopressin-like peptides in a variety of species (for reviews see: Dierickx 1980; Buijs 1987; Korf et al. 1988). Most of the neurosecretory cells are located in hypothalamic nuclei that are mainly involved in the well-known hypothalamo-neurohypophysial system. More recently, it has been established that in addition to this hormonal system, an extensive vasopressin-like fiber network, arising inside and outside the hypothalamus, also projects to extrahypothalamic regions in the brain, thus suggesting a neuromodulator and neurotransmitter function. In fish, the location of vasotocin-immunoreactive systems has only been investigated in teleost species (Goossens et al. 1977a; Schreibman and Halgner 1980; van den Dungen et al. 1982; Yulis and Lederis 1987; Olivereau et al. 1988). These studies have demonstrated that immunoreactive cell bodies are concentrated in the preoptic area; vasotocin-containing neurons project through the basal hypothalamus to the pituitary. Only in the trout, *Salmo gairdneri*, have some immunoreactive fibers also been detected outside the hypothalamus (van den Dungen et al. 1982).

To our knowledge, no study has been undertaken to determine the location of vasotocin-containing neurons in the brain of elasmobranchs.

The present investigation reports findings of immunocytochemical studies aimed at characterizing the distribution of vasotocin-like substances in the brain and pituitary of the elasmobranch fish, *Scyliorhinus canicula*. The possible coexistence of vasotocin- and CRF-like im-

monoreactivity in the same neurons has also been investigated.

Materials and methods

For the present study 12 mature male and female dogfish (*Scyliorhinus canicula*) were used. The animals were captured by trawl net in the Ligurian sea (Western Mediterranean). They were fished in June and kept in filtered aerated aquaria at a constant temperature ($13 \pm 0.5^\circ\text{C}$), and under natural light-dark cycles, for 1 week. The animals were first anesthetized by putting them in a small tank containing tricaine methanesulfonate (MS 222, Sandoz; 100 mg/liter seawater) and then perfused through the ventral aorta with cold saline-phosphate buffer (PBS) (0.05 M; pH 7.4). The perfusion was subsequently continued with either a solution of 4% paraformaldehyde in PBS, or alternatively, with Bouin's fixative. The brains, with the attached pituitary, were removed and post-fixed overnight in the respective fixative solutions. Bouin-fixed brains were dehydrated in ethanol, cleared in xylene and embedded in paraffin; serial 5- μm -thick sections were cut in the transverse and sagittal planes. Paraformaldehyde-fixed brains were transferred into a PBS-20/30% sucrose solution for 24 h at 4°C ; the brains were frozen on dry-ice and 20- μm -thick sections were cut in a cryostat (-18°C). The sections were then processed by either an immunofluorescence technique (Coons et al. 1955) or by the peroxidase-antiperoxidase technique (PAP) (Sternberger 1979).

The sections processed for immunofluorescence labelling were first treated with normal swine serum (Dakopatts, DK) (0.7 mg/ml) for 20 min, then incubated at 4°C for 18 h in a moistened chamber with anti-vasotocin serum, provided by Dr. D.A. Gray, Bad Nauheim, FRG (Gray and Simon 1983). The antiserum was raised in rabbits against synthetic vasotocin, and was diluted 1:1200 with a 0.3% Triton X-100/PBS solution containing 1% human serum albumin. Alternate serial sections were treated with ovine anti corticotropin-releasing factor serum (CRF) (UCB, Belgium), to check possible coexistence with vasotocin in the same perikarya. Thereafter, the sections were thoroughly rinsed in several baths of PBS and incubated for 1 h at room temperature in fluorescein isothiocyanate-conjugated swine anti-rabbit gammaglobulins (Dakopatts, DK) diluted 1:100 in PBS. Finally, the sections were washed 3 times in PBS and mounted in a glycerol-PBS (1:1) solution and examined under a Zeiss epifluorescence microscope.

The sections processed for the PAP method were treated with vasotocin antiserum, diluted 1:1200, as described above. After being washed in PBS, the slides were treated with swine antiserum to rabbit gammaglobulins (Dakopatts, DK; diluted 1:500) for 1 h at room temperature, and then with rabbit peroxidase-antiperoxidase complex (Dakopatts, DK; diluted 1:400) for a further hour. The antigens were visualized using 3,3'-diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.01% hydrogen peroxide in PBS solution. Alternate sections were counterstained with cresyl violet to determine the localization of the immunopositive structures.

In order to check the specificity of the immunoreaction, controls were subjected to immunohistochemical procedures in which one of the steps was omitted, or by preabsorbing the primary antisera with synthetic homologous peptides (Bachem, Budendorf, Switzerland) (10^{-6}M). In some experiments the primary antiserum was replaced by non-immunized rabbit serum or PBS. Nomenclature used in this study to describe the anatomical regions of the brain was based on the work of Smeets et al. (1983).

Results

General distribution of vasotocin immunoreactivity

Cell bodies and nerve fibers containing vasotocin-like immunoreactivity were found in the hypothalamus of

all examined specimens. No specific immunopositive products were observed in consecutive sections treated with antiserum preabsorbed with the homologous antigen or with the normal non-immunized rabbit serum. We have not characterized the cross-reactivity of the antiserum against isotocin (a nonapeptide of the same family which is present in other fishes) because older data have demonstrated that this peptide is not present in the elasmobranchs (Matty 1985).

Vasotocin-like immunoreactive perikarya were detected primarily throughout the mid-caudal region of the preoptic nucleus. The most rostral immunoreacting elements were located in the dorso-lateral parts of the medial preoptic area (Fig. 1). Proceeding more caudally the neurons took a ventromedial position. They were densely packed within the deepest layers of the nucleus, and formed a continuous population extending to the suprachiasmatic level, with the main concentration located towards the caudal part of the preoptic recess. Figure 1 also shows the reported distribution of corticotropin-releasing factor (CRF)-positive cells recently described in the preoptic nucleus of the same cartilaginous fish (Vallarino et al. 1989a). These cells occur in the anterior and medial parts of the preoptic nucleus, generally located in the subependymal layers (Figs. 1a-c). In the medial part of the nucleus, which contains both CRF and vasotocin-immunoreactive cells, they are located in mid position, along the ventral walls of the preoptic recess. This location of CRF cells, was also confirmed in the present study in the adjacent serial sections of the preoptic nucleus which were treated with anti vasotocin- and anti CRF-sera, respectively. The rostral vasotocin-like immunostained cells were intensively reactive and showed fine processes with a caudo-latero-ventral direction (Fig. 2). At more caudal levels the immunoreactive cells were ventro-medially arranged, closely packed to form clusters within the inner component of the preoptic nucleus (Fig. 3). Occasionally, scattered vasotocin-like immunostained cells were identified in the postchiasmatic region within the nucleus periventricularis hypothalami. These cells were small in size and sparsely distributed in the dorsal wall of the periventricular area (Fig. 4).

Vasotocin immunoreactivity was observed in numerous varicose nerve fibers of the hypothalamo-hypophysial tract. These fibers, originating from the preoptic nucleus, formed a dense bundle which, after having passed the lateral chiasmatic region, coursed caudally in the medio-basal hypothalamus, parallel to the floor of the brain. Numerous stained fibers were seen to penetrate the caudal hypothalamus (Fig. 5). They passed through the thin hypophysial stem to reach the neurointermediate lobe of the pituitary, in which a high density of fibers and terminals were located within its rostral region (Fig. 6). A large component of the fiber bundle reached the median eminence along its rostro-medial region. Within the median eminence the fibers made close contacts with the capillary loops (Figs. 7, 8). No immunoreactive structures were found in other brain regions.

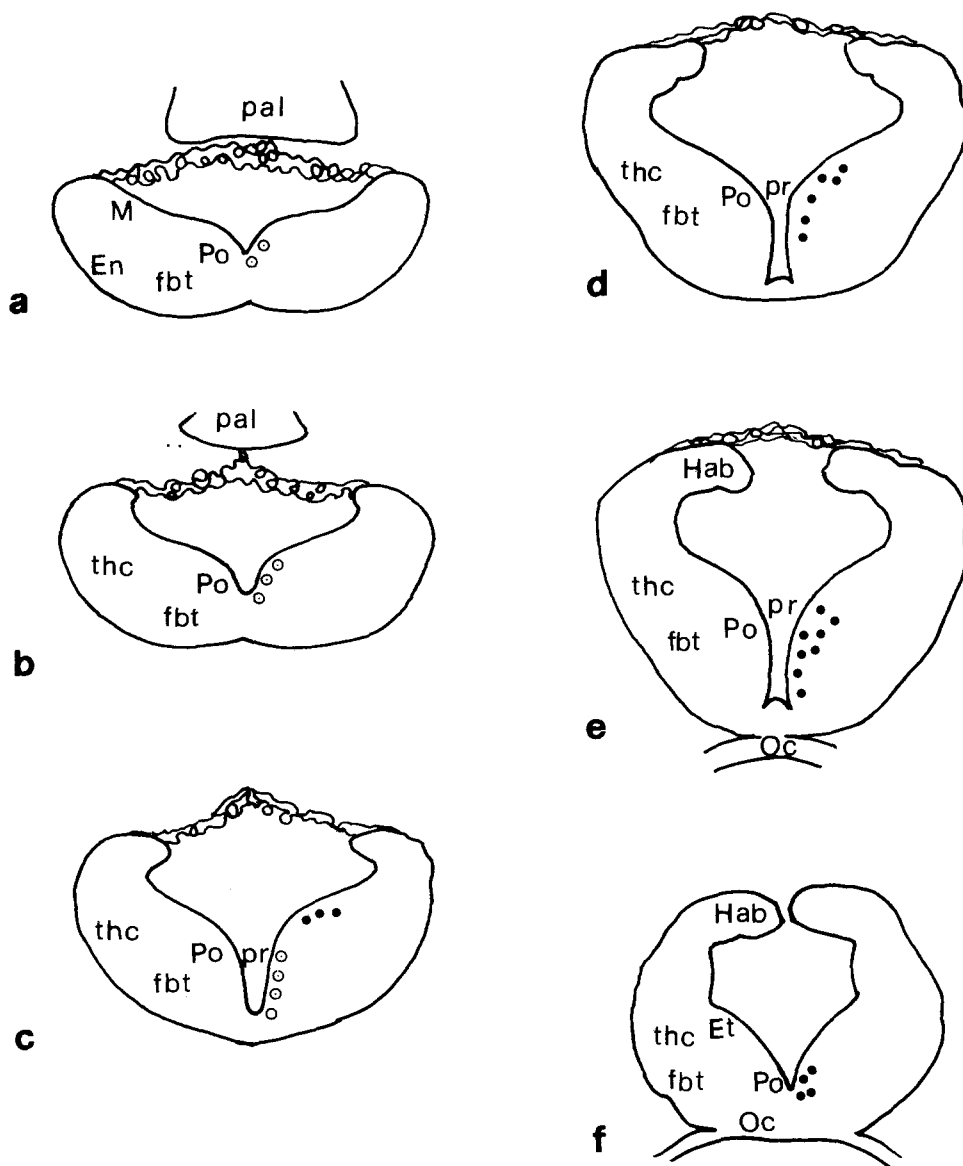


Fig. 1. Schematic transverse sections through the rostral hypothalamus of *Scyliorhinus canicula* at the level of the preoptic nucleus (progressing from the anterior section **a**, to the caudal section **f**), showing the distribution of immunoreactive CRF- (○) and vasotocin-containing (●) perikarya. *En* nucleus entopeduncularis, *Et* eminentia thalami, *fbt* fasciculus basalis telencephali, *Hab* ganglion habenulae, *M* nucleus M, *Oc* chiasma opticum, *pal* pallium, *Po* nucleus praeopticus, *pr* recessus praeopticus, *thc* tractus thalamocorticalis of Johnston

Morphology of the preoptic vasotocin neurons

Throughout the preoptic nucleus the vasotocin-immunoreactive cells varied in shape according to their location. The most rostral neurons were oval, generally with a thick, long process emerging from the ventral side of the cell body; branchings were also observed close to the perikarya. Some laterally scattered cells showed end branchings that appeared to contact non-reactive cells. The cells located in the caudal part of the preoptic nucleus were more frequently round in shape, with short, large and intensely stained, processes. Occasionally, the immunoreactive neurons appeared bipolar in shape (Figs. 9–12). No CSF-contacting immunoreactive cell type was found in our material.

Discussion

This study provides evidence of a localization of vasotocin-like immunoreactive substances in the brain of the elasmobranch fish, *Scyliorhinus canicula*. The immunoreactive cells and fibers are located in the hypothalamus, within the preoptico-hypophysial system, and occasionally in the nucleus periventricularis hypothalami. The presence of such immunoreactivity in the preoptico-hypophysial complex is in agreement with previously reported data in several species of teleosts (Goossens et al. 1977a; Schreibman and Halgner 1980; van den Dungen et al. 1982; Yulis and Lederis 1987; Olivereau et al. 1988), in the lungfish *Protopterus aethiopicus* (Goossens et al. 1978), and in the cyclostome *Lampetra fluviatilis*

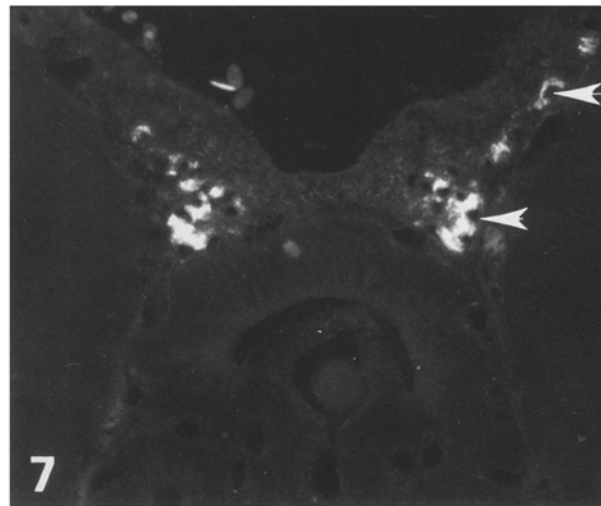
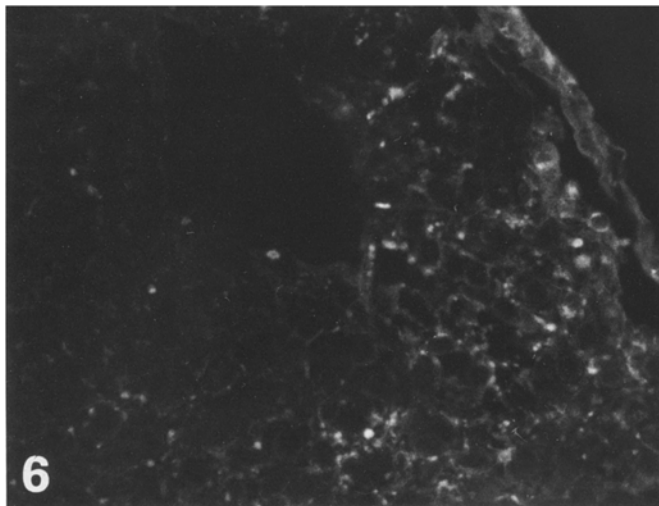
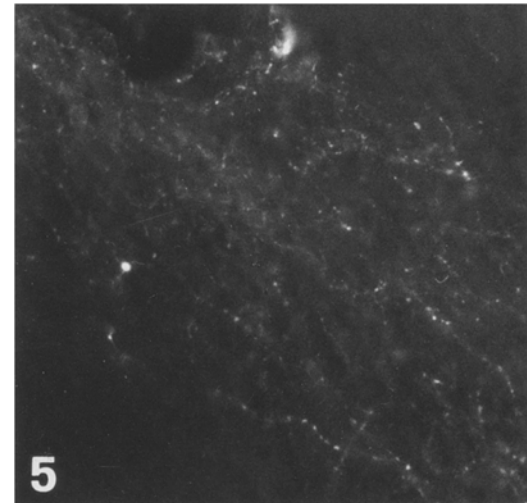
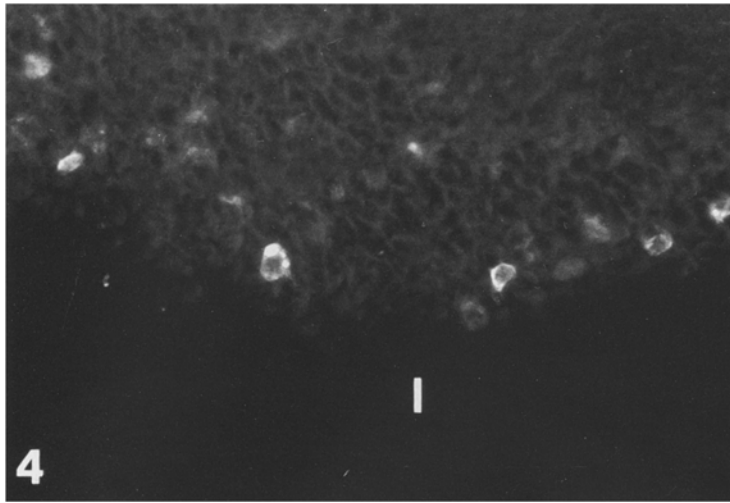
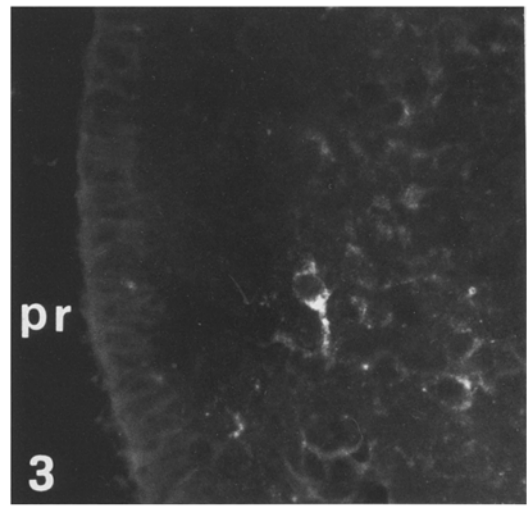
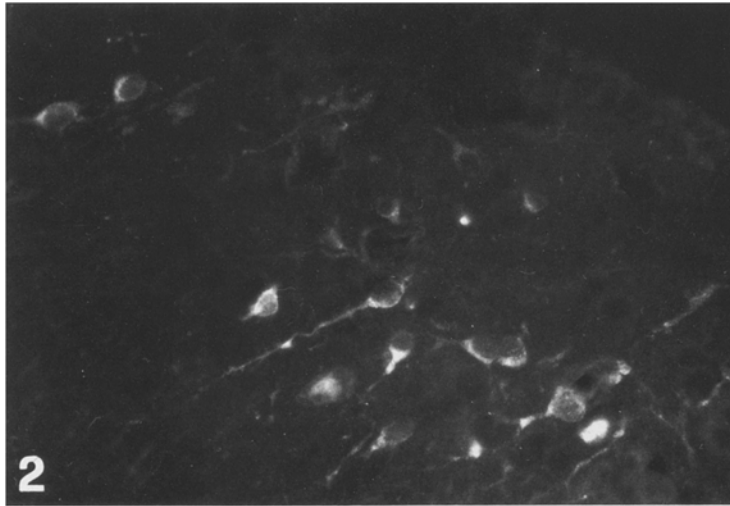


Fig. 2. Vasotocin-like containing neurons in a transverse section of the medial preoptic nucleus revealed by immunofluorescence. $\times 340$

Fig. 3. Fluorescence photomicrograph showing vasotocin-like perikarya within the mid-caudal part of the preoptic nucleus; *pr* preoptic recess. $\times 340$

Fig. 4. Frozen sagittal section through the nucleus periventricularis hypothalami showing immunofluorescence vasotocin-like neurons. *I* infundibulum. $\times 340$

Fig. 5. Paraffin sagittal section of the basal hypothalamus showing immunofluorescence vasotocin-like beaded fibers. $\times 340$

Fig. 6. Frozen sagittal section showing vasotocin-like immunoreactive fibers within the rostral part of the pituitary neurointermediate lobe. $\times 340$

Fig. 7. Paraffin transverse section through the hypothalamus showing bright fluorescent fibers within the median eminence. The *arrows* indicate some vascular structures surrounded by vasotocin-like reactive fibers. $\times 125$

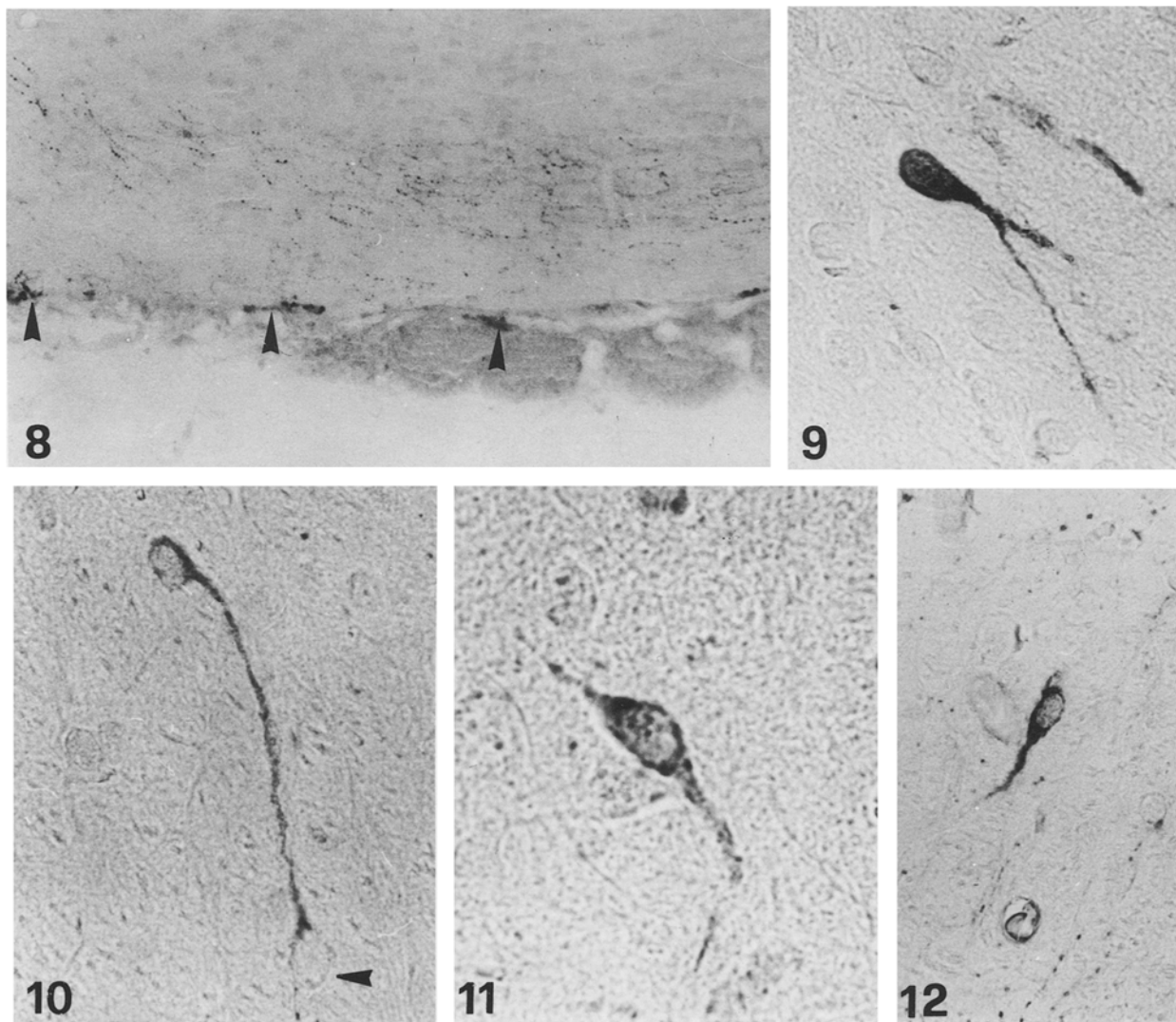


Fig. 8. Paraffin sagittal section of the medial hypothalamus showing numerous immunoreactive beaded fibers in the preoptico-hypophysial tract. The *arrows* indicate stained fibers ending in the median eminence. PAP immunoreaction. $\times 125$

Figs. 9–12. These microphotographs show typical vasotocin-like immunoreactive cells localized in the preoptic nucleus. PAP immunoreaction.

Fig. 9. Perikarya oval in shape showing branchings close to the cell bodies. $\times 340$. **Fig. 10.** A positive neuron with a process that appears to contact a non-reactive cell (*arrow*). $\times 340$. **Figs. 11 and 12** show respectively a bipolar neuron and a small classical element of the caudal preoptic area. $\times 340$

(Goossens et al. 1977b). The occurrence of immunoreactive elements in the nucleus periventricularis hypothalami, however, represents the first findings of vasotocin-like neurons outside the preoptic area in fish.

Compared with the published data on tetrapods, the distribution of vasotocinergic cells and fibers in fishes appears to be less diffuse. In all vertebrates, the main population of vasopressin/vasotocin-immunoreactive neurons is located in the preoptic-rostral hypothalamic region, and in higher vertebrates (e.g., mammals, birds and reptiles), these neurons are organized into more or less recognizably distinct nuclei (the classical supraoptic and paraventricular nuclei (Korf et al. 1988)). The situation is, however, rather different in amphibians and fishes, where this rostral system is not differentiated in easily distinguishable nuclei.

As well as the hypothalamic population of neurons, immunoreactive cells and fibers have also been observed in extrahypothalamic sites in different tetrapods. In mammals, for example, vasopressin-immunoreactive cells have been reported to be localized in the bed nucleus of the stria terminalis, the locus coeruleus, and the amygdala (for a detailed literature cf. Buijs 1987); immunoreactive fibers are also distributed in several regions of the brain stem as far as the medulla and spinal cord (Sofroniew 1985). In birds, vasotocin immunoreactive neurons have been observed in the rostral mesencephalon (Panzica et al. 1988), while positive fibers have been detected throughout the whole brain stem to the nucleus of the solitary tract (Panzica et al. 1986, 1988). In reptiles, positive cells have been described in the bed nucleus of the stria terminalis and in the rhombencephalon,

whereas immunopositive fibers have been found in all brain regions (Stoll and Voorn 1985; Thepen et al. 1987). In amphibians, immunopositive fibers have been found outside the hypothalamus, in many brain regions, such as the limbic cortex, thalamus, optic tectum and lower brain stem (Zoeller and Moore 1986; Jokura and Urano 1987). In fishes, the occurrence of extrahypothalamic vasotocin fibers has, to date, only been reported in the trout *Salmo gairdneri* (van den Dungen et al. 1982).

In the present study, vasotocin-containing neurons have been observed in precise regions of the preoptic nucleus, a well developed neurosecretory area that merges rostrally within the telencephalic floor, lines the preoptic recess, and more caudally extends to the dorsal optic chiasma (Mazzi 1952; Perks 1969). The neurosecretory properties of the preoptic nucleus in *Scyliorhinus* have been described by Mazzi (1952), Scharrer (1952), and Bargmann (1953), and recently we have demonstrated that it contains different subpopulations of perikarya producing NPY- (Vallarino et al. 1988), CRF- (Vallarino et al. 1989, a) beta-endorphin- (Vallarino et al. 1989, b), and bombesin-like substances (Vallarino et al. 1990). These peptidergic cells are mainly located in the rostral region of the nucleus, with the exception of the bombesin-immunoreactive neurons which are distributed throughout the whole rostro-caudal length. Since the vasotocin-immunoreactive cells are located in the mid-caudal region of the preoptic nucleus, the possible coexistence of vasotocin with the other peptides in the same neuronal population would appear to be rather unlikely. Colocalization of vasotocin- and CRF-like immunoreactivity has been reported in the preoptic nucleus of teleosts (Yulis and Lederis 1987; Olivereau et al. 1988), and it is matter of controversy in birds (see for a review Korf et al. 1988). In mammals, vasopressin and CRF may coexist in experimental conditions (adrenalectomized animals) in the paraventricular parvocellular neurons (Roth et al. 1982; Tramu et al. 1983; Sawchenko et al. 1984; Kiss et al. 1984; Piekut and Joseph 1986). In *Scyliorhinus canicula*, on the other hand, we have not found evidence of coexistence between vasotocin- and CRF-immunoreactive neurons because of their location at different levels of the preoptic nucleus. Besides, most of the CRF-positive cells are of the CSF-contacting type and are distributed in the subependymal layers lining the ventricle, whereas the vasotocin-like elements do not show these peculiarities.

The present study has also demonstrated that the preoptic nucleus contains various cellular types producing vasotocin-like substances. Most of these send their long fibers in the preoptico-hypophysial tract toward the median eminence and the neurointermediate lobe of the pituitary, whereas some may also be involved in short cell-to-cell contacts as indicated by stained fibers that appear close to non-reactive cells in the preoptic area. This latter observation, if confirmed at the electron-microscopic level, might suggest a vasotocin neuromodulator function in fish. This hypothesis has previously been suggested for other vertebrate species, in which vasotocin fibers appear to end within the preoptic region and are probably involved in the control of sexual

behaviour (Moore and Miller 1983; Urano 1988). The presence of vasotocin-like immunoreactivity in the neurointermediate lobe of the pituitary confirms previous pharmacological data in the same species (Heller 1941; Perks and Dodd 1963), which also demonstrated the existence of high concentrations of oxytocin-like activity.

Little is known about the physiological role of vasotocin in elasmobranchs. To date there is no clear evidence for a role in water or salt balance (Perks 1969; Dodd et al. 1966; Henderson et al. 1988), but the possibility of a local effect within the pituitary has been suggested (Perks 1969). The particular hypophysial vascular system present in many species of elasmobranchs, or the extensive penetration of the pars nervosa with the pars intermedia, as in *Scyliorhinus*, may support the hypothesis of an involvement of neurohypophysial hormones in melanotropic activities (Mellinger 1963). The present finding of an extensive distribution of immunoreactive fibers close to the vascular structures of the median eminence, suggests that vasotocin may also function as an hypophysiotropic agent in the pars distalis, as reported in teleosts where vasotocin is able to stimulate gonadotropic and corticotropic cells (Groves and Batten 1986; Fryer et al. 1985).

In conclusion, our results show that the general pattern of distribution of vasotocinergic elements in a representative species of elasmobranch is largely comparable with the few existing reports for other piscine species. It seems that in this more primitive fish group there is a reduction in the extent of the extrahypothalamic fiber system. Even if limited, it appears that some of the fibers are not involved in the hypothalamo-neurohypophysial system (some of them may terminate within the same preoptic nucleus or at the level of the median eminence), thus suggesting several functional roles for this system.

Acknowledgements. This research was supported by a grant from the Italian M.P.I. (40%). The authors gratefully acknowledge the photographic assistance provided by Mr. R. Marini.

References

- Acher R (1985) The non-mammalian-mammalian transition through neurohypophysial peptides. *Peptides* 6 [Suppl 3]: 309–314
- Bargmann W (1953) Über das Zwischenhirn-Hypophysensystem von Fischen. *Z Zellforsch Mikrosk Anat* 38:275–298
- Buijs RM (1987) Vasopressin localization and putative functions in the brain. In: Gash DM, Boer GJ (eds) *Vasopressin: principles and properties*. Plenum Press, New York, pp 91–115
- Coons AH, Leduc EH, Conolly JM (1955) Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. *J Exp Med* 102:49–60
- Dierickx K (1980) Immunocytochemical localization of the vertebrate cyclic nonapeptide neurohypophysial hormones and neurophysins. *Int Rev Cytol* 62:119–185
- Dodd MHJ, Perks AM, Dodd MHI (1966) Physiological functions of neurohypophysial hormones in submammalian vertebrates. In: Harris GW, Donovan BT (eds) *The pituitary gland*. Butterworth, London Washington, vol 3, pp 578–623
- Dungen HM van den, Buijs RM, Pool CW, Terlouw M (1982) The

- distribution of vasotocin and isotocin in the brain of the rainbow trout. *J Comp Neurol* 212:146–157
- Fryer J, Lederis K, Rivier J (1985) ACTH-releasing activity of urotensin I and ovine CRF: interactions with arginine vasotocin, isotocin and arginine vasopressin. *Reg Pept* 11:11–15
- Goossens N, Dierickx K, Vandesande F (1977a) Immunocytochemical localization of vasotocin and isotocin in the preoptico-hypophysial neurosecretory system of teleosts. *Gen Comp Endocrinol* 32:371–375
- Goossens N, Dierickx K, Vandesande F (1977b) Immunocytochemical demonstration of the hypothalamo-hypophysial vasotocinergic system of *Lampetra fluviatilis*. *Cell Tissue Res* 177:317–323
- Goossens N, Dierickx K, Vandesande F (1978) Immunocytochemical study of the neurohypophysial hormone producing system of the lungfish, *Protopterus aethiopicus*. *Cell Tissue Res* 190:69–77
- Gray DA, Simon E (1983) Mammalian and avian antidiuretic hormone: studies related to possible species variation in osmoregulatory systems. *J Comp Physiol* 151:241–246
- Groves DJ, Batten TFC (1986) Direct control of gonadotrophin in a teleost, *Poecilia latipinna*. II. Neurohormones and neurotransmitters. *Gen Comp Endocrinol* 62:315–326
- Heller H (1941) The distribution of the pituitary antidiuretic hormone throughout the vertebrate series. *J Physiol* 99:246–256
- Henderson IW, O'Toole LB, Hazan N (1988) Kidney function. In: Shuttleworth TJ (ed) *Physiology of elasmobranch fishes*. Springer, Berlin Heidelberg New York, pp 201–214
- Kiss JZ, Mezey E, Skirboll L (1984) Corticotropin-releasing factor-immunoreactive neurons of the paraventricular nucleus become vasopressin positive after adrenalectomy. *Proc Natl Acad Sci USA* 81:1854–1858
- Korf HW, Panzica GC, Viglietti-Panzica C, Oksche A (1988) Pattern of peptidergic neurons in the avian brain: clusters-local circuitries-projections. *Basic Appl Histochem* 32:55–75
- Jokura Y, Urano A (1987) Extrahypothalamic projection of immunoreactive vasotocin fibers in the brain of the toad, *Bufo japonicus*. *Zool Sci* 4:675–681
- Matty AJ (1985) *Fish endocrinology*. Croom Helm, London Sydney, Timber Press Portland, Oregon, pp 15–28
- Mazzi V (1952) I fenomeni neurosecretori nel nucleo magnocellulare preottico dei selaci e dei ciclostomi. *Riv Biol* 44:429–449
- Mellinger JCA (1963) Etude histophysiologique du système hypothalamo-hypophysaire de *Scyliorhinus caniculus* (L) en état de melanodispersion permanente. *Gen Comp Endocrinol* 3:26–45
- Moore FL, Miller LJ (1983) Arginine vasotocin induces sexual behavior of newts by acting on cells in the brain. *Peptides* 4:97–102
- Oliverau M, Moons L, Oliverau J, Vandesande F (1988) Coexistence of corticotropin-releasing factor-like immunoreactivity and vasotocin in perikarya of the preoptic nucleus in the eel. *Gen Comp Endocrinol* 70:41–48
- Panzica GC, Fiori MG, Viglietti-Panzica C (1986) Vasotocin fibers in the mesencephalon and pons of the domestic fowl. An immunohistochemical study. *Neurosci Lett* 68:155–159
- Panzica GC, Calcagni M, Ramieri G, Viglietti-Panzica C (1988) Extrahypothalamic distribution of vasotocin immunoreactive fibers and perikarya in the avian central nervous system. *Basic Appl Histochem* 32:89–94
- Perks AM (1969) The neurohypophysis. In: Hoar WS, Randall DJ (eds) *Fish Physiology*, vol II. Academic Press, New York San Francisco London, pp 111–205
- Perks AM, Dodd MHJ (1963) The properties of the oxytocin, milk ejection and antidiuretic principle of the neurointermediate lobe of the elasmobranch pituitary. *Gen Comp Endocrinol* 3:184–195
- Piekut DT, Joseph SA (1986) Co-existence of CRF and vasopressin immunoreactivity in parvocellular paraventricular neurons of rat hypothalamus. *Peptides* 7:891–898
- Roth KA, Weber E, Barchas JD (1982) Immunoreactive corticotropin-releasing factor (CRF) and vasopressin are colocalized in a subpopulation of the immunoreactive vasopressin cells in the paraventricular nucleus of the hypothalamus. *Life Sci* 31:1857–1860
- Sawchenko PE, Swanson LW, Vale WW (1984) Co-expression of corticotropin-releasing factor and vasopressin immunoreactivity in parvocellular neurosecretory neurons of the adrenalectomized rat. *Proc Natl Acad Sci USA* 81:1883–1887
- Scharrer E (1952) Das Hypophysen-Zwischenhirnsystem von *Scylium stellare*. *Z Zellforsch* 37:196–204
- Schreibman MP, Halgner LR (1980) The demonstration of neurophysin and arginine vasotocin by immunocytochemical methods in the brain and pituitary gland of the platyfish, *Xiphophorus maculatus*. *Gen Comp Endocrinol* 40:1–7
- Smeets WJAJ, Nieuwenhuys R, Roberts BL (1983) *The central nervous system of cartilaginous fishes*. Springer, Berlin Heidelberg New York
- Sofroniew MW (1985) Vasopressin- and neurophysin-immunoreactive neurons in the septal region, medial amygdala and locus coeruleus in colchicine-treated rats. *Neurosci* 15:347–358
- Sternberger LA (1979) *Immunocytochemistry*. 2nd ed, Wiley, New York, pp 104–169
- Stool CJ, Voorn P (1985) The distribution of hypothalamic and extrahypothalamic vasotocinergic cells and fibers in the brain of a lizard *Gekko gekko*; presence of a sex difference. *J Comp Neurol* 239:193–204
- Thepen TH, Voorn P, Stoll CJ, Sluiter AA, Pool CW, Lohman AHM (1987) Mesotocin and vasotocin in the brain of the lizard *Gekko gekko*. An immunocytochemical study. *Cell Tissue Res* 250:649–656
- Tramu G, Croix C, Pillez A (1983) Ability of the CRF immunoreactive neurons of the paraventricular nucleus to produce a vasopressin-like material. *Neuroendocrinology* 37:467–469
- Urano A (1988) Neuroendocrine control of anuran anterior preoptic neurons and initiation of mating behavior. *Zool Sci* 5:925–937
- Vallarino M, Danger JM, Fasolo A, Pelletier G, Saint-Pierre S, Vaudry H (1988) Distribution and characterization of neuropeptide Y in the brain of an elasmobranch fish. *Brain Res* 448:67–76
- Vallarino M, Fasolo A, Ottonello I, Perroteau I, Tonon MC, Vandesande F, Vaudry H (1989a) Localization of corticotropin-releasing hormone (CRF)-like immunoreactivity in the central nervous system of the elasmobranch fish, *Scyliorhinus canicula*. *Cell Tissue Res* 258:541–546
- Vallarino M, Tranchand Bunel D, Delbende C, Ottonello I, Vaudry H (1989b) Distribution of the pro-opiomelanocortin-derived peptides, alpha-melanocyte-stimulating hormone (a-MSH), adrenocorticotrophic hormone (ACTH) and beta-endorphin in the brain of the dogfish *Scyliorhinus canicula*. An immunocytochemical study. *J Exp Zool* S2:112–121
- Vallarino M, D'Este L, Negri L, Ottonello I, Renda T (1990) Occurrence of bombesin-like immunoreactivity in the brain of the cartilaginous fish, *Scyliorhinus canicula*. *Cell Tissue Res* 259:177–181
- Yulis CR, Lederis K (1987) Co-localization of the immunoreactivities of corticotropin-releasing factor and arginine vasotocin in the brain and pituitary system of the teleost *Catostomus commersoni*. *Cell Tissue Res* 247:267–273
- Zoeller RT, Moore FL (1986) Arginine vasotocin immunoreactivity in hypothalamic and extrahypothalamic areas of an amphibian brain. *Neuroendocrinology* 42:120–123