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Comparative localization of corticotropin and corticotropin releasing factor-like peptides in the brain and hypophysis of a primitive vertebrate, the sturgeon *Acipenser ruthenus L.*

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Summary

The sturgeon is a primitive actinopterigian fish that, unlike modern teleosts, possess a portal vascular system that connects a true median eminence with the anterior pituitary as in mammals. The occurrence and localization of corticotropin and corticotropin releasing factor-like immunoreactivities were examined in the brain of the sturgeon (*Acipenser ruthenus L.*) by immunocytochemistry with antisera raised against synthetic non-conjugated human corticotropin, and rat/human corticotropin releasing factor. In the hypothalamus, corticotropin-immunoreactive parvicellular perikarya were found in the infundibular nucleus and in dendritic projections to the infundibular recess. In addition, ependymofugal corticotropin-immunoreactive fibres were found to terminate in the ventral hypothalamus. Corticotropin releasing factor-immunoreactive neurons were found in the rostral portion of the ventral hypothalamus (tuberal nucleus), and in the vicinity of the rostral aspect of the lateral recess. These cells projected to the dorsal hypothalamus, the ventral hypothalmus, the median eminence, the anterior and posterior telencephalon, the tegmentum mesencephali, and the pars nervosa of the pituitary. An affinity-purified UI antiserum failed to stain the sturgeon hypothalamus. Corticotrophs in the rostral pars distalis of the pituitary were also corticotropin-immunoreactive. In the neurointermediate lobe, only about 50% of cells of the pars intermedia appeared to be corticotropin-positive, the rest appeared unstained. These results suggest that the presence of corticotropin-like and corticotropin releasing factor-like peptides in the brain is a relatively early event in vertebrate evolution, already occurring in Chondrostean/Actinopterigian fishes, as exemplified by *A. ruthenus*.

The close spatial relationship between corticotropin releasing factor immunoreactivity and corticotropin immunoreactivity in the ventral hypothalamus of *A. ruthenus* supports a possible interaction between the two systems in that area of the sturgeon brain. The pars intermedia might be an important site for corticotropin synthesis, even though the possibility cannot be excluded that the antiserum was recognizing the proopiomelanocortin molecule. The occurrence of corticotropin releasing factor immunoreactivity in the region of median eminence/pars intermedia of the sturgeon suggests that the sturgeon corticotropin releasing factor might regulate the adenohypophyseal release of proopiomelanocortin products in the same manner as in other vertebrates. The presence of extrahypothalamic corticotropin releasing factor-immunoreactive projections suggests further neuromolulatory functions for this peptide in *A. ruthenus*.

Introduction

The occurrence of proopiomelanocortin (POMC) has been demonstrated in the pituitary of primitive actinopterigian and amphibian vertebrates (Hansen *et al.*, 1980; Hansen, 1983; Joss *et al.*, 1990; see also Dores, 1990), and the end products of this precursor adrenocorticotropin (ACTH) and melanocyte-stimulating hormone (MSH) have been chemically characterized in the spiny dogfish, (*Squalus acanthias*) (Lowry *et al.*, 1974), the chum salmon (*Oncorhynchus keta*) (Kawauchi, 1983), *Xenopus laevis* (Martens, 1986), and *Rana catesbeiana* (Yasuda *et al.*, 1989). The presence of ACTH, α-MSH and β-endorphin has been demonstrated by

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immunocytochemistry in the pituitary of various teleostome and elasmobranchiomorph fishes (see Baker, 1980). Radioimmunoassayable ACTH, α-MSH, and β-endorphin have been reported in the rainbow trout *Salmo gairdneri* (Swift, 1982; Bowley *et al.*, 1983; Takahashi *et al.*, 1984; Rodrigues & Sumpter, 1984; Vallarino *et al.*, 1989), and in the chinook salmon *Oncorhynchus tschawytscha* (Sumpter & Donaldson, 1986).

In the mammalian brain POMC derivatives are also present in the perikarya of the arcuate nucleus, the nucleus of the solitary tract, and the zona incerta (Liotta & Krieger, 1983; Krieger, 1983; Tranchand-Bunel *et al.*, 1987; Smith & Funder, 1988). Similarly, POMC derivatives were identified in the brains of several teleost species including *Carassius auratus* (Bonn & Köning, 1988), *S. gairdneri* and *Ctenopharyngodon idellus* (Kishida *et al.*, 1988; Bird & Baker, unpublished results), and amphibian species such as *R. esculenta* (Vaudry *et al.*, 1978), *R. ridibunda* (Benyamina *et al.*, 1986), *R. catesbeiana* and *R. pipiens* (González *et al.*, unpublished results).

In vitro and *in vivo* studies in mammals (Vale *et al.*, 1983; Rivier & Plotsky 1986; Labrie *et al.*, 1987) and non-mammalian species (Lederis, 1987; Verburg-Van Kemenade *et al.*, 1987; Fryer, 1989; Tran *et al.*, 1990) demonstrated that hypothalamic corticotropin-releasing factor (CRF) is one of the main regulators of the synthesis and release of POMC derivatives from the pituitary.

In mammals, the CRF-immunoreactive (ir) perikarya that project to the median eminence and that affect ACTH release are located in the paraventricular nucleus (Fellmann *et al.*, 1984; Sawchenko & Swanson, 1985; Sakanaka *et al.*, 1987). In the brains of the teleost fish *Catostomus commersoni* (Yulis *et al.*, 1986) and bullfrog *R. catesbeiana* (González & Lederis, 1988) two different CRF-ir neuronal populations have been described. In the fish (*C. commersoni*) the two separated CRF-ir group of cells are localized in the preoptic nucleus (the phyletic predecessor of the supraoptic and paraventricular nuclei of mammals) and the nucleus lateralis tuberis (Yulis *et al.*, 1986), whereas in the bullfrog the two CRF-ir cell groups are localized in the preoptic nucleus, albeit in different subpopulations of neurons (González & Lederis, 1988).

The distribution of POMC and CRF peptides in the brain of actinopterigian vertebrates has not been completely investigated. Among these vertebrates, the sturgeons (Chondrostei, Actinopterigii) represent a primitive group because they are considered to occupy a position close to the main stream of vertebrate evolution (Severtsov, 1967) and near to the branch point of the teleost and sarcopterigean lineages.

In contrast to a more modern actinopterigian fish, the sturgeons have a true median eminence separated from the pars distalis by a thin connective tissue stratum, a portal vascular system that supplies the pars distalis, and a neurohypophysis which is an interdigitation of the infundibulum into the pars intermedia (Gorbman *et al.*, 1982; Batten, 1985; see also Fig. 22).

These distinctive aspects of the brain-pituitary relationship as well their crucial phylogenetic position make the sturgeons an important group to study for the understanding of the phylogeny of neuropeptidergic systems of the hypothalamo-hypophyseal axis. In the present work we investigated the immunocytological distribution of ACTH and CRF in the brain and hypophysis of *A. ruthenus*, by using specific antisera raised against non-conjugated mammalian $ACTH_{11-24}$ and CRF. Preliminary results from ACTH staining were reported earlier (González *et al.*, 1989).

Materials and methods

Sexually mature sturgeons (*Acipenser ruthenus*) of both sexes captured in the delta of the Volga river (Russia) in June 1987 were used in the present study. The fishes were killed by decapitation and the brains including the hypophysis were dissected out of the skull and immersed in complete Bouin's fluid for 4 h followed by a post-fixation in Bouin's without acetic acid for 48 h. Paraffin-embedded serial sections cut in the coronal and sagittal planes were mounted on gelatin-coated slides. Before immunostaining, the sections were treated with xylene, hydrated, and exposed to 3–10% H_2O_2 to reduced endogenous peroxidase activity.

Immostaining was performed according to the peroxidase antiperoxidase (PAP) procedure as modified by Sofroniew and Glassman (1981).

Corticotropin antiserum (2A2) was raised in rabbits in our laboratory against synthetic non-conjugated $ACTH_{11-24}$. The rationale behind the use of the antigen $ACTH_{11-24}$ was that the recognition of the $ACTH_{11-24}$ antiserum should preclude recognition of α-MSH ($ACTH_{1-13}$). The primary sequence of $ACTH_{11-24}$ is highly conserved among the vertebrates (see Baker, 1980; Chang *et al.*, 1980; Kawauchi, 1983; Martens, 1986; Yasuda *et al.*, 1989). In addition, residues between aminoacids 11–24 are considered to be important in binding the hormone to its receptor site (Baker, 1980) which implies that this region is exposed to the solvent and so may participate in antigen–antibody binding. Therefore, we anticipated that the recognition of an antigenic determinant with structural homology to the known ACTHs would not be altered. Consequently, in radioimmunoassay (RIA), the 2A2 antiserum crossreacted with $ACTH_{11-24}$, $ACTH_{18-39}$, and $ACTH_{1-24}$, but not with α-MSH ($ACTH_{1-13}$) (Fryer, personal communication). Corticotropin releasing factor antiserum (1C4) was raised in rabbits against synthetic non-conjugated ovine (oCRF) and rat/human CRF (r/h CRF). In RIA, the antiserum crossreacted with oCRF and r/h CRF (100%), but only minimally with sucker (*C. commersoni*) urotensin I (sUI 0.23%) and sauvagine (0.009%) (Lederis *et al.*, 1987).

The urotensin I antiserum (4D1) was raised against non-conjugated sucker UI. In RIA it crossreacted with sUI (100%) but only minimally (<0.15%) with oCRF (Lederis, *et*

al., 1987). Immunocytological studies (Yulis *et al.*, 1986; Yulis & Lederis, 1986) indicated that this antiserum could recognize CRF brain structures in the rat and fish. After purification by solid phase adsorption of the CRF antibodies (Yulis & Lederis, 1986) the UI antiserum (p4D1) failed to stain the aforementioned CRF structures but stained UI-ir perikarya in the urophysis of the sucker. We used this purified UI (p4D1) antiserum in the present study.

Tissue sections were incubated with the primary antisera (1:500) diluted in Tris-phosphate buffer saline (T-PBS: 1.18 g Na_2HPO_4, 0.43 g KH_2PO_4, 7 g NaCl, 5 g Trizma, 0.2 g NaN_3 in 1 l of distilled water, and adjusted to pH 7.8), containing 0.4% Triton X-100 (Sigma), and 0.6% non-gelling carrageenan (Sigma), for 18 h, at room temperature. Triton X-100 and carrageenan were used to facilitate the penetration of the antibodies into the tissue and to reduce non-specific binding (Sofroniew & Glassman, 1981). Then, the sections were incubated with goat antirabbit IgG (Sigma, 1:25, for 30 min), and PAP (Dako, 1:50, for 30 min), diluted in T-PBS but containing only 0.1% Triton X-100. Finally, the sections were developed for 15 min, in the dark, with 0.2% diaminobenzidine (Sigma) solution in the same buffer containing 0.005% H_2O_2.

Specificity tests were done by incubating adjacent sections with the primary antiserum solution previously immunoabsorbed with 10 μM (final concentration of the peptide) of $ACTH_{1-24}$, $ACTH_{11-24}$, $ACTH_{18-39}$, α-MSH, or somatostatin (2A2 antiserum), and with sUI, r/h CRF or $ACTH_{1-24}$ (1C4 antiserum) (Table 1).

Results

Immunocytochemistry of the sturgeon brain

Numerous bipolar or polygonal nerve cells, 15–25 μm in diameter, were immunostained in the caudal aspect of the ventral hypothalamus (infundibular nucleus) (Figs 2, 4, 6). In a coronal view, the ACTH-ir neurons appeared located either close to the ependyma or in the ependymal layer lining the floor and the ventral parts of the lateral walls of the third ventricle. They extended to the area surrounding the infundibular recess (Figs 2, 6). In a parasagittal view, the ACTH-ir cells could be seen dorsal to the tissue stratum separating the proximal pars distalis from the floor of the hypothalamus (Figs 6, 22). Rather thick dendrites of the ACTH-ir neurons could be followed towards the ventricular lumen (Figs 11, 12). The beaded axons from ACTH-ir perikarya appeared to terminate only in the ventral hypothalamus among CRF-ir perikarya and projections (Figs 4, 22). No ACTH-ir terminals were seen in the median eminence or in the neurohypophysis (Figs 2, 4, 6, 10, 22).

The CRF-ir cells (Figs 1, 22) however were located in the rostral part of the ventral hypothalamus caudal to the posterior border of the chiasmatic field. The cells occupied a position similar to the nucleus lateralis tuberis of the more evolved teleost fishes. We have called this localization the tuberal nucleus, to differentiate it from the more caudal (infundibular) ACTH-ir neurons in the hypothalamus (see Fig. 22).

Corticotropin releasing factor-immunoreactive fibres were seen not only in the ventral hypothalamus and median eminence (Figs 17, 22), but also in other brain areas including the dorsal aspect of the lateral recess (Figs 16, 22), and an area below the ventral aspect of the posterior recess (Fig. 14). Other CRF-ir projections could be followed coursing from the forebrain bundle over the chiasmatic ridge and into the anterior preoptic area (Figs 19, 22) and also in the anteroventral (Figs 21, 22), and posterdorsal (Figs 20, 22) telencephalon. Finally, CRF-ir fibres were also observed in the tegmentum mesencephali (Figs 18, 22). No ACTH- or CRF-ir cells were seen in the preoptic nucleus.

In the pituitary, numerous CRF-ir terminals were present in the pars nervosa of the neurointermediate lobe, close to the ACTH-ir cells of the pars intermedia (Figs 13, 22).

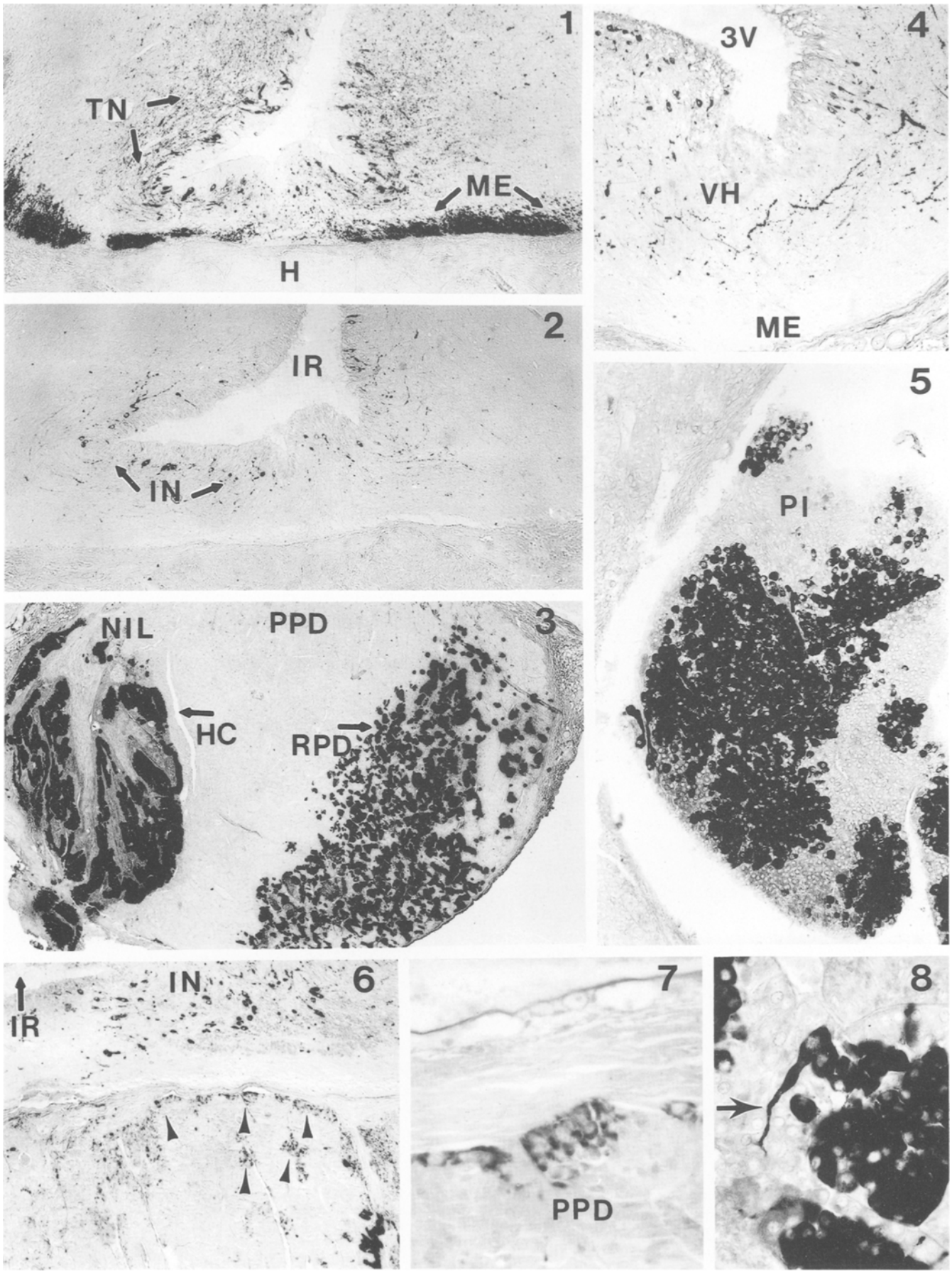
Immunocytochemistry of the sturgeon adenohypophysis

Specific and intensive immunocytochemical reaction with the antiserum against $ACTH_{11-24}$ was seen in two regions of the sturgeon pituitary, the dorsal and ventral parts of the rostral par distalis and the pars intermedia (Figs 3, 5, 8, 10, 22). Oval, pear-shaped or prismatic (10 × 20 μm) ACTH-ir cells with large eccentrically located nuclei were stained along the connective tissue septa and secondary portal capillaries supplying the hypophyseal pars distalis (Fig. 9). The cytoplasm of these cells was filled with intensively immunoreactive granules. Clusters of ACTH-ir cells

Table 1. Effects of immunoabsorption with different peptides (10 μM in all cases) prior to immunostaining with the ACTH and CRF antisera.

Antiserum	*$ACTH_{1-24}$*	*$ACTH_{11-24}$*	*$ACTH_{18-39}$*	*α-MSH*	*r/h CRF*	*sUI*	*SRIF*
2A2 (ACTH)	–	–	–	+	+	ND	+
1C4 (CRF)	+	ND	ND	ND	–	+	ND

– Abolition of staining
\+ No change in immunostaining
ND Not done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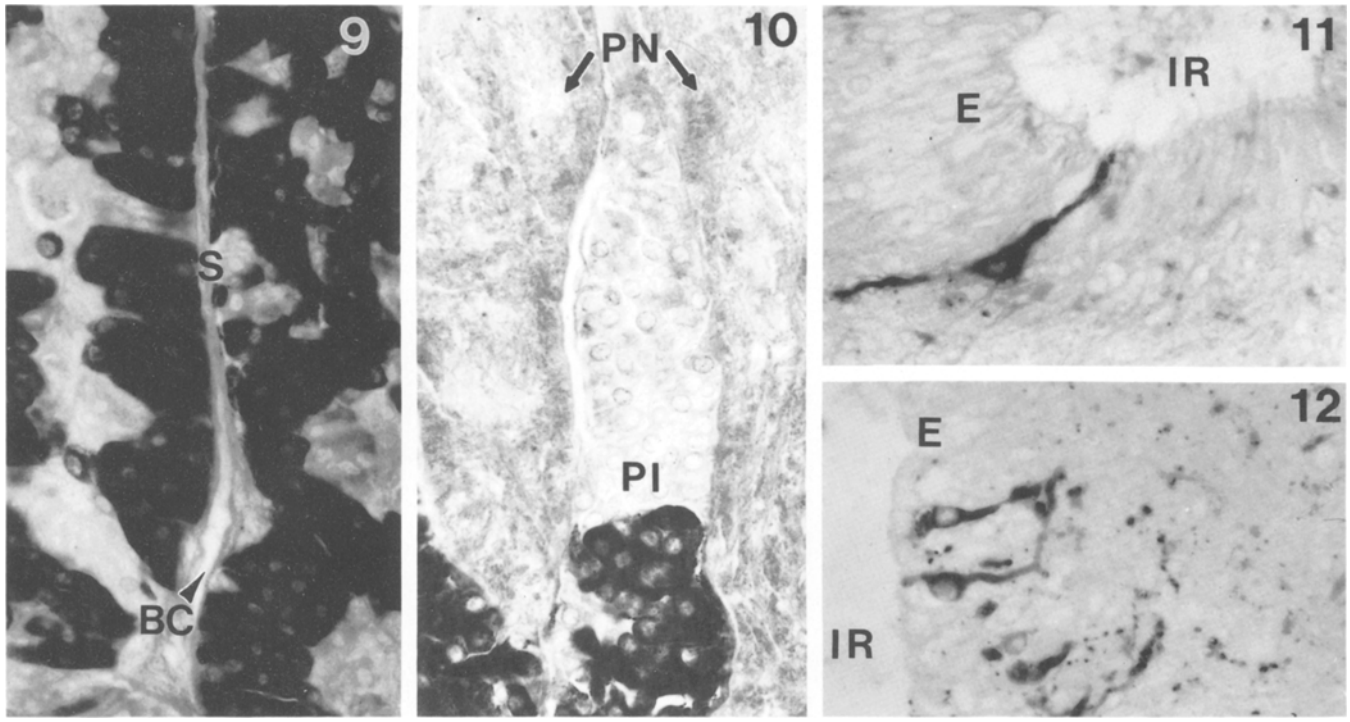


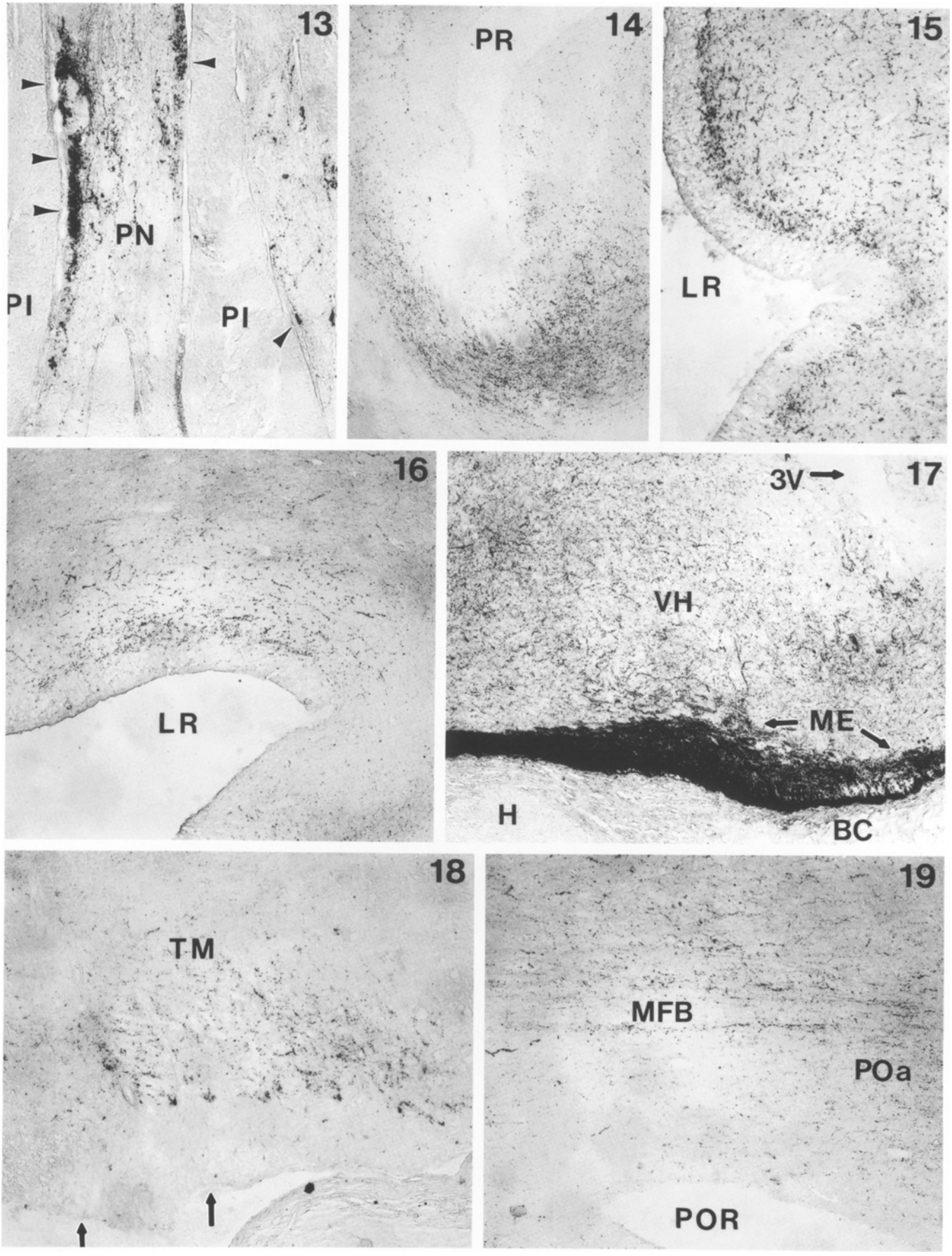
Figs 9–12. Higher magnification photomicrographs showing in some detail ACTH-ir in neurons of IN, as well as in glandular cells of the hypophysis of *A. ruthenus*. (9) Sagittal section through the rostral pars distalis showing ACTH-producing cells close to thin connective tissue septa (S). × 400. (10) Coronal section through the NIL, showing ACTH-ir staining in part of the PI cells and no immunostaining in the pars nervosa. Corticotropin-immunoreactive glandular cells are located close to the connective tissue septa separating the PI from roots of the pars nervosa. Note that the PI immunonegative cells are similar to the ACTH-ir cells. × 406. (11) Corticotropin-immunoreactive cell with a long dendrite protruding into the ependyma and opening into the third ventricle. × 400. (12) Higher magnification of coronal section through the caudo-ventral hypothalamus of the sturgeon showing ACTH-ir cell bodies with short dendritic projections and terminal bouton in the ependymal layer. × 400.

were also found in the dorsal part of the proximal pars distalis, mainly near the connective tissue stratum separating the pituitary from the brain tissue (Figs 6, 7).

In the neurointermediate lobe, only about 50% of the pars intermedia cells showed distinct ACTH immunostaining (Fig. 5). A great number of these ACTH-ir glandular cells were seen close to the connective tissue septa separating the pars intermedia from the pars nervosa (Fig. 10). Others were in the deeper

Figs 1–14. Abbreviations for Figs 1–14 are as follows: AVT, anteroventral telencephalon; BC, blood capillaries; C, cerebellum; E, ependyma; H, hypophysis; HC, hypophyseal cleft; IN, infundibular nucleus; IR, infundibular recess of the third ventricle (3V); LR, lateral recess of the third ventricle; ME, median eminence; MFB, medial forebrain bundle; NIL, neurointermediate lobe; ON, optic nerve; OT, optic tectum; PDT, posterodorsal telencephalon; PI, pars intermedia; PN, pars nervosa; POa, anterior preoptic area; POR, preoptic recess of the third ventricle; PPD, proximal pars distalis; PR, posterior recess of the third ventricle; RPD, rostral pars distalis; S, connective tissue septa; SV, saccus vasculosum; TM, tegmentum mesencephali; TN, tuberal nucleus; VH, ventral hypothalamus.

Figs 1–8. Photomicrographs showing ACTH-ir or CRF-ir in the brain and pituitary of the sturgeon *Acipenser ruthenus*. (1) Coronal section through the ventral hypothalamus immunostained for CRF. Note the great array of CRF-ir fibres in the ME. × 100. (2) Section caudal to previous one and immunostained for ACTH. Note the absence of immunostaining in the ME. × 100. (3) Low magnification micrograph of a sagittal section through the hypophysis showing ACTH-ir glandular cells in the pars intermedia of the NIL and RPD (corticotrophs). × 40. (4) Parasagittal section showing some ACTH-ir perikarya and beaded fibres restricted to the rostroventral hypothalamus. × 100. (5) Photomicrograph of an oblique section passing through the PI. Note that the $ACTH_{11-24}$ antiserum (2A2) stains only about half of the PI cells. × 104. (6) Parasagittal section showing ACTH-ir neurons in the caudoventral hypothalamus (IN), just above the tissue stratum separating the PPD from the floor of the hypothalamus. Note the ACTH-ir cells in the most dorsal part of the RPD (lower right corner), and also the ACTH-ir cell clusters (arrowheads) in the dorsal region of the proximal pars distalis (PPD). × 100. (7) Higher magnification of ACTH-ir cells cluster appearing in the PPD. × 400. (8) Detail showing a glandular cell in the pars intermedia with a thin cytoplasmic protrusion (arrow). × 400.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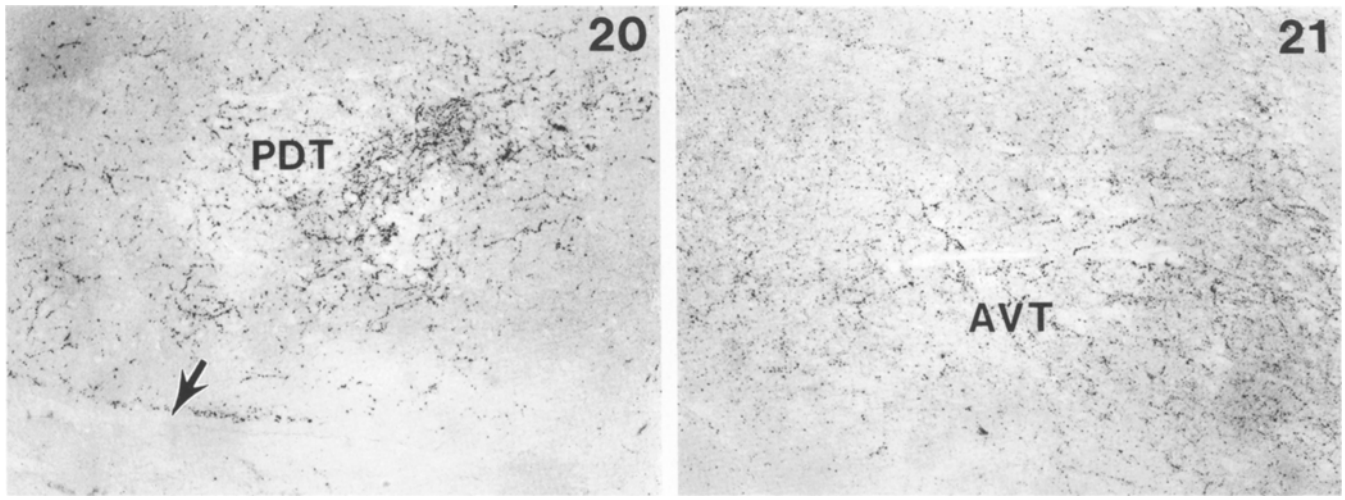

Figs 20–21. Corticotropin releasing factor immunoreactivity fibres in the telencephalon of *A. ruthenus*. (20) Posterodorsal telencephalon. The arrow indicates the posterior border of the cerebral hemisphere. × 100. (21) Anteroventral telencephalon. × 100.

layers of the glandular parenchyma (Fig. 8). A few of the ACTH-ir cells appeared to extend thin cytoplasmic protrusions into the glandular parenchyma (Fig. 8). The unstained glandular cells of the pars intermedia had an appearance and structure (Figs 5, 8, 10) similar to the ACTH-immunopositive cells.

Figure 22 summarizes the distribution of CRF-like and ACTH-like peptides in the brain and pituitary of the sturgeon *A. ruthenus*.

Preincubation of the anti-ACTH serum with α-MSH, r/h CRF, or SRIF did not affect the intensity and distribution of immunostaining either in the brain or in the different parts of the pituitary. In contrast, absorption with $ACTH_{1-24}$, $ACTH_{11-24}$ and $ACTH_{1-39}$ completely prevented the immunostaining (Table 1). Immunostaining with the CRF (1C4) antiserum was abolished by preincubation of the primary antiserum with synthetic r/h CRF. Heterologous blockage with $ACTH_{1-24}$ or sucker urotensin I (sUI) did not prevent the immunostaining (Table 1).

A urotensin I antiserum (p4D1) previously purified by solid phase adsorption of CRF antibodies (Yulis & Lederis, 1986), failed to give positive staining in the sturgeon brain.

Discussion

This study demonstrates the presence of numerous intensely stained ACTH-ir nerve cells in the caudal hypothalamus, in an area corresponding to the infundibular nucleus, the phyletic predecessor of the arcuate nucleus of amniote vertebrates. In addition we demonstrate CRF-ir perikarya in the tuberal nucleus of the sturgeon rostral hypothalamus. The immunostained perikarya and projections either indicate the presence of ACTH and CRF peptides in the CNS of the sturgeon, or the presence of immunologically related substances that share epitopes with mammalian ACTH or CRF.

Although the amino acid sequence of sturgeon ACTH is not known, its similarity to mammalian ACTH is suggested by our findings as well as by the evidence indicating that ACTH has been highly conserved during the evolution of vertebrates (see Baker, 1980; Chang *et al.*, 1980; Kawauchi, 1983; Martens, 1986; Yasuda *et al.*, 1989). Immunoabsorption of the ACTH 2A2 antiserum suggests that antibodies present in the ACTH antiserum probably recognize the mid-portion and/or the C-terminal portion of the putative

Figs 13–19. Photomicrographs of parasagittal sections showing the distribution of CRF-ir projections in the brain of *A. ruthenus*. (13) Pars nervosa. Note that most of the CRF-ir terminals are adjacent to the connective tissue septa between PN and PI (arrowheads). × 160. (14) Fibres in the ventral part of the posterior recess. × 100. (15) Fibres in rostral aspect of lateral recess. × 100. (16) Fibres in the dorsal aspect of the lateral recess. × 100. (17) Ventral hypothalamus and median eminence. Compare the number and intensity of the CRF-ir fibres shown in this figure with the smaller number of ACTH-ir fibres in the ventral hypothalamus shown in Fig. 4. × 100. (18) Tegmentum mesencephali. The arrows indicate the ventral pial surface of the mesencephalon. × 100. (19) Fibres coming through the medial forebrain bundle (MFB) over the chiasmic ridge and penetrating into the anterior preoptic area (POa). × 100.

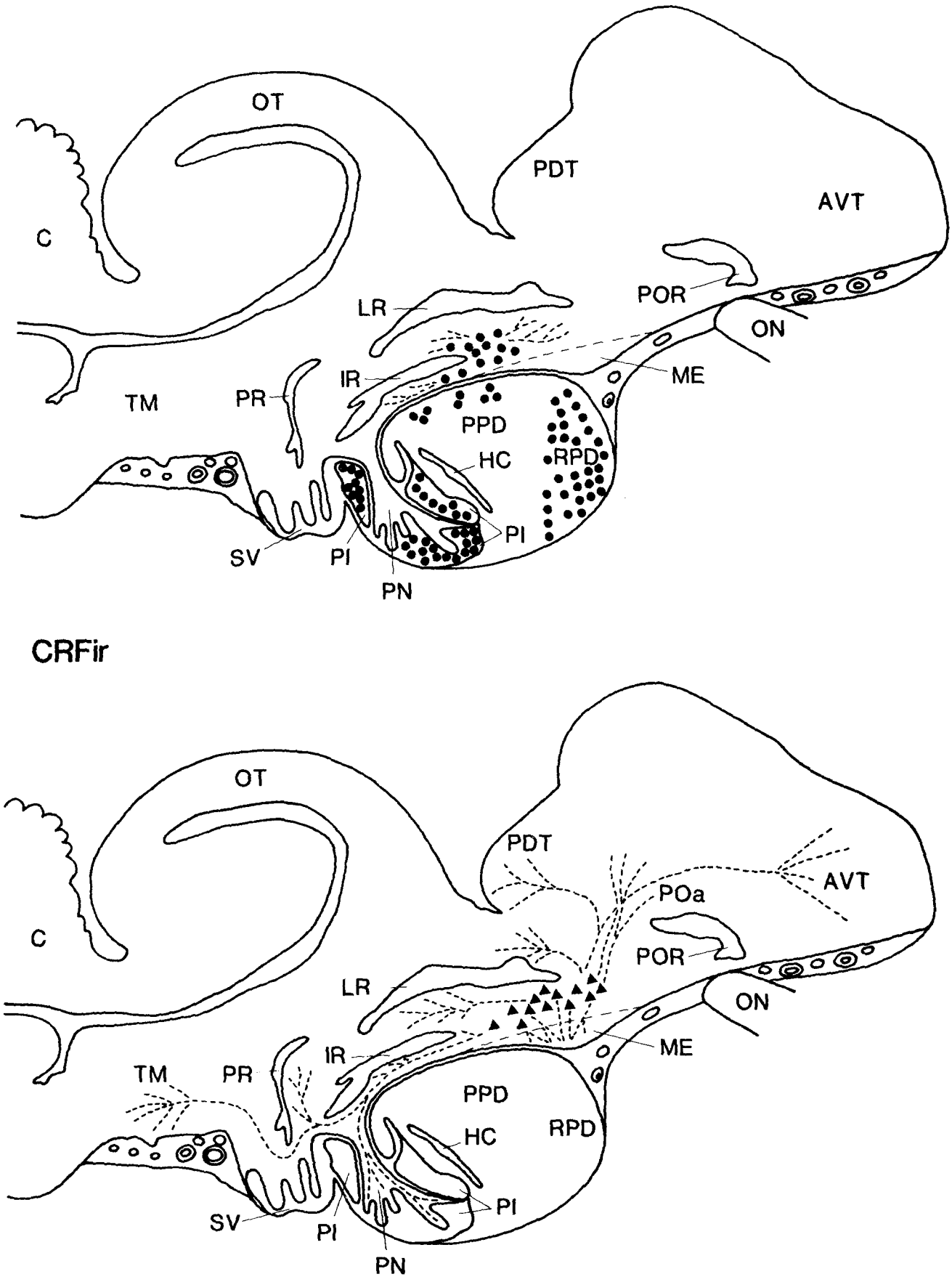

Fig. 22. *Schematic parasagittal representation of ACTH (upper figure) and CRF (lower figure) immunoreactivity distribution in the sturgeon (A. ruthenus) brain and hypophysis. The symbols do not represent the quantitative distribution of the appropriate cell type. Rostral is to the right. Closed circles: ACTH-ir cells. Closed triangles: CRF-ir cells. Dashed lines: immunoreactive fibres (ACTH-ir and CRF-ir).*

sturgeon ACTH, but not of α-MSH. Immunostaining in presumptive α-MSH localization (e.g. pars intermedia of the sturgeon) was abolished by preincubation with $ACTH_{11-24}$ suggesting the presence of ACTH in these cells.

Similar ACTH-ir has been observed in the ventral hypothalamus of the rainbow trout *S. gairdneri* and grass carp *C. idellus* (Bird & Baker, unpublished results) and of the goldfish *C. auratus* (Bonn & König, 1988). In the rainbow trout and grass carp, neurons in this area were also immunostained by α-MSH antiserum, suggesting that both ACTH and α-MSH immunoreactivities may coexist in the same cells (Kishida *et al.*, 1988). In the lamprey (*Entosphenus tridentatus*), β-endorphin and Met-enkephalin-like immunoreactivity were found in the basal hypothalamus, but no positive reaction was found after application of anti-ACTH serum (Nozaki & Gorbman, 1984). In contrast, Eastman and Portanova (1982) reported low bioassayable ACTH activity in the brain of *Lampetra aepyptera*, and Baker and Buckingham (1983) showed, by using RIA, an MSH-like immunoreactivity in the brain of *Lampetra fluviatilis*.

It is salient to note that a pattern of ACTH-ir similar to that seen in the sturgeon was also found in the infundibular nucleus of the frogs *R. pipiens* and *R. catesbeiana* (González, Yulis & Lederis, unpublished results) and in the infundibular region of the white-crowned sparrow *Zonotrichia leucophrys gambeli* (Blähser, 1988). Neurons immunoreactive for ACTH, α-MSH, and β-endorphin have been described in the arcuate nucleus and nucleus of the solitary tract of the mammalian brain (see Tranchard-Bunel *et al.*, 1987). Peptides of the POMC family have been detected in the hypothalamic region of mammalian brain by HPLC and RIA (see Smith & Funder 1988). A group of α-MSH immunoreactive neurons was observed also in the zona incerta localized in the dorso-lateral hypothalamus of mammals (Tranchard-Bunel *et al.*, 1987).

The observation of ACTH-ir 'ventricular' dendrites located among the ependymal cells of the sturgeon suggests that an ACTH-like peptide may be discharged into the cerebrospinal fluid. Whether this peptide affects subependymal CRF-ir perikarya and projections bordering the III ventricle and infundibulum remains speculative.

The glandular cells located in the upper part of the rostral pars distalis which were intensively immunostained with the anti-$ACTH_{11-24}$ may be viewed as the ACTH-producing cells of the sturgeon pituitary. A similar localization of ACTH cells was noted in the pituitary of *A. transmontanus* (Hansen, 1983), as well as in several teleostean species (see Follénius *et al.*, 1978; Naito *et al.*, 1984; Cambré *et al.*, 1986; Moons *et al.*, 1988; Quesada *et al.*, 1988).

Corticotropin-immunostained cells in the rostral pars distalis of *A. ruthenus*, like those of *A. transmontanus* (Hansen, 1983) are closely associated with the blood capillaries of the secondary portal plexus. In most teleostean fishes a hypophyseal portal system is lacking, and the ACTH-producing cells are characteristically located in the rostro-dorsal part of the pars distalis close to the roots of the anterior neurohypophysis, where the neurosecretory axons 'innervating' these cells are situated (Olivereau *et al.*, 1984; Moons *et al.*, 1988; Fryer, 1989). All these findings demonstrate a dependence of the functional activity of ACTH-producing cells of the rostral pars distalis on hypothalamic control.

The immunostaining of the peripheral region of some dorsally located cells of the proximal pars distalis seen in the sturgeon *A. ruthenus* was not detected in *A. transmontanus* pituitary (Hansen, 1983). However immunoreactivity was shown in the homologous region of the adenohypophysis of some teleost fishes with the use of anti-$ACTH_{17-39}$ serum (Follénius *et al.*, 1978). It remains to be decided whether in the proximal pars distalis of Acipenseridae the cells reacting with anti-$ACTH_{11-24}$ contain an immunologically related substance with or without biological ACTH activity.

Approximately 50% of the cells from the sturgeon (*A. ruthenus*) pars intermedia were immunoreactive for $ACTH_{11-24}$. However, no ACTH-immunopositive cells were seen in this part of the pituitary in *A. transmontanus* by using a commercial anti-$ACTH_{1-24}$ serum (Hansen, 1983). This discrepancy may be related to antibody affinity and to the sensitivity of the immunocytochemical procedure. In a variety of teleostean species ACTH and MSH immunoreactivities co-exist in the same cells of the pars intermedia (Follénius *et al.*, 1978; Naito *et al.*, 1984; Cambré *et al.*, 1986; Quesada *et al.*, 1988). These findings are in line with the hypothesis that both hormones are derived from a common precursor molecule.

The present study suggests that in the sturgeon the pars intermedia may also be an important site for ACTH secretion. However, it cannot be excluded that our ACTH antiserum may recognize an intact POMC molecule.

When alternate sections through the hypothalamic region of the sturgeon were stained for ACTH or CRF, no overlap of the two staining patterns occurred, suggesting that the two peptides are produced by different cells. The distinct distributions of axonal projections ACTH-ir and CRF-ir neurons also favour this view. Our present results with an antiserum raised against r/h CRF (1C4 antiserum), as well as previous data obtained after immunostaining with two different anti-ovine CRF antisera (Belenky *et al.*, 1985) show CRF-ir fibres terminating in the median eminence. In the present study, we did not see ACTH-ir terminals in the median eminence of the sturgeon. Similarly, ACTH staining was not observed in a teleostean fish (Bonn & König, 1988; Kishida *et al.*,

1988), nor in the median eminence of some mammalian and other non-mammalian species (Sofroniew, 1979; Knigge & Joseph, 1981; Blähser, 1988). In addition, while the ACTH-ir fibre distribution in the sturgeon was restricted to the ventral hypothalamus, the CRF-ir perikarya sent projections to other brain areas such as the telencephalon, dorsal hypothalamus, and mesencephalon. In keeping with the findings of Belenky and colleagues (1985), we also found CRF-ir fibres terminating in the digitations of the pars nervosa that abut the pars intermedia. The latter observation supports recent evidence suggesting that CRF may be one of the neural agents regulating the release of POMC products from pars intermedia in anamniotes (Verburg-van Kemenade *et al.*, 1987; Fryer, 1989; Tran *et al.*, 1990; Dores, 1990).

With the use of our 1C4 antiserum, CRF-ir perikarya were revealed exclusively in the tuberal nucleus of the sturgeon hypothalamus. Immunostained cells had also been detected previously, by using two anti-ovine CRF sera, in the preoptic nucleus of the sturgeon (Belenky *et al.*, 1985). This discrepancy in the results may relate to varying specificities of the antisera, and is consistent with the existence of two different populations of CRF-like cells in the preoptic nucleus and nucleus lateralis tuberis of the teleost *C. commersoni* (Yulis *et al.*, 1986), and in the preoptic nucleus of the bullfrog *R. catesbeiana* (González & Lederis, 1988). We did not find CRF-ir neurons in the preoptic nucleus of *A. ruthenus*. The CRF-ir perikarya in the tuberal nucleus of the sturgeon may correspond to the CRF-ir perikarya in the nucleus lateris tuberis of more modern actinopterigian fishes.

In addition to demonstrating CRF-ir cells in the preoptic nucleus, the use of an affinity purified urotensin I (UI) antiserum (p4D1) has shown that UI-ir neurons are also present in the parvicellular region of the nucleus lateralis tuberis of *C. commersoni* (Yulis & Lederis, 1986). The latter UI-ir cells could not be demonstrated in the sturgeon when the same purified antiserum was used. This discrepancy suggests that a CRF-like peptide (possibly with two different epitopic structures) may exist in the brain of the sturgeon and that the infundibular CRF-ir might share closer structural homology either with sucker (*C. commersoni*) CRF, porcine CRF or r/h CRF (see Lederis *et al.*, 1990), than with ovine CRF.

In conclusion, as in mammals ACTH-ir and CRF-ir cells are present in the CNS of the sturgeon *A. ruthenus* L., a primitive chondrostean fish which is considered to occupy the nearest position among Actinopterigian fishes to the mainstream of vertebrate evolution. Corticotropin immunoreactive cells in the infundibular nucleus, and CRF-ir neurons in the tuberal nucleus support the view that brain POMC/CRF may have appeared early in vertebrate evolution. The strongly stained CRF-ir terminals in the median eminence suggest that CRF may exert its effect on the corticotrophs via the vasculature relating the median eminence to the rostral pars distalis. In addition, the presence of extrahypothalamic CRF-ir fibres suggests additional neuromodulatory functions for CRF in the CNS of the sturgeon. The absence of ACTH-ir fibres in the median eminence or pars nervosa suggests that the site of action of hypothalamic ACTH may be within the brain. In the pituitary, the pars distalis and the pars intermedia appear to be important sites for the synthesis of ACTH.

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