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Immunocytochemical identification of CRF-like and SRIF-like peptides in the brain and the pituitary of cyprinid fish

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Summary. Single and double immunocytochemical techniques were applied to the brain and pituitary of carps and goldfish. With the use of antiserum raised against synthetic corticotropin-releasing factor (CRF), immunoreactive perikarya were observed in the nucleus praeopticus and the nucleus praeopticus periventricularis. CRF-like-immunoreactive hypothalamic nerve fibers reach the pituitary. In cyprinids, some fine fibers enter the rostral neurohypophysis bordered by prolactin- and ACTH cells. Other thicker fibers extend ventrocaudally into the neurointermediate lobe. This CRF-like system appears to differ from the SRIF-like system, which is restricted to the proximal pars distalis of the pituitary containing somatotrophs.

Key words: Corticotropin-releasing factor (CRF) – Somatostatin (SRIF) – Brain – Preoptic nucleus – Pituitary gland – Teleosts

A 41-amino acid peptide that stimulates secretion of corticotropin and β-endorphin (Vale et al. 1981) has been isolated from ovine hypothalami. With the use of immunocytochemistry it has been demonstrated that the external region of the mammalian median eminence contains nerve fibers showing a CRF-like immunoreactivity. After intracisternal or intraventricular colchicine injections, large numbers of perikarya containing a CRF-like substance were observed in the paraventricular nucleus (Bugnon et al. 1982; Hashimoto et al. 1982; Tramu and Pillez 1982; Swanson et al. 1983)

The chemical composition of CRF in non-mammalian vertebrates is unknown. However, in *Salmo gairdneri*, nerve fibers containing a CRF-like-immunoreactive material have been described to occurr in the neurohypophysis (NH). A group of fibers is located in the rostral NH, facing the corticotropic (ACTH) cells in the rostral pars distalis (RPD). Other bundles of fibers occur in the caudal NH, at the level of the neurointermediate lobe (NIL) (Bugnon et al. 1983; Ollevier and Verdonck 1984). The two groups of CRF-like-immunoreactive fibers do not mingle with the SRIF (somatostatin)-containing fibers present in the proximal pars distalis (PPD) (Ollevier and Verdonck 1984). CRF-like-immunoreactive cell bodies are observed in the preoptic nucleus (PON) (Bugnon et al. 1983; Verdonck and Ollevier 1984). Most fish do not possess a hypothalamo-hypophysial portal system, and their median eminence is not similar to that of mammals. LHRH and SRIF have been localized in some areas of the rostral neurohypophysis. As ACTH cells and somatotrops (GH cells) show a constant and precise location in the pituitary of teleosts, it is important to investigate the relation between certain cell types and their respective releasing or inhibiting factors. The present paper reports on data obtained in two cyprinid species.

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Materials and methods

Twenty goldfish, *Carassius auratus* L. (3–4 g BW), and six carps, *Cyprinus carpio* L. (375 g BW), were adapted at 20±1° C to a 12L:12D photoperiod. Brains and pituitaries were fixed in sublimated Bouin-Hollande solution and embedded in paraffin. Immunocytochemical stainings were performed on 4-μm thick sections.

Antisera to synthetic CRF 1–41, somatostatin 1–28, ACTH 1–24 and isotocin, all raised in rabbits, were tested at a dilution of 1/1000 and 1/2000. Immunoreactive peptides were revealed by the PAP method (peroxidase-antiperoxidase technique) either with single PAP staining (3,3′-diaminobenzidine) (DAB), or double PAP staining (DAB and 4-chloro-1-naphthol) (Vandesande et al. 1977). The demonstration of CRF-like-immunoreactive material was coupled to that of ACTH or SRIF. Tests for specificity of the methods and antisera were performed as previously described (Vandesande et al. 1977) by omitting the primary antiserum or using a primary antiserum exhausted by solid phase immunoadsorption on a Sepharose 4 B antigen-complex. In both cases, the reaction was negative.

Results and discussion

CRF-like-immunoreactive perikarya were observed in the nucleus praeopticus periventricularis and the PON, mainly in the parvocellular area (Fig. 1). They were scattered or arranged in small clusters mainly in the dorsal area of the two nuclei. The perikarya are triangular or occasionally bipolar in shape. They show a large nucleus (average diameter: 6–7 μm; maximal diameter; 10 μm) and a small amount of cytoplasm containing fine and larger immunoreactive granules. The processes of the labeled cells are rarely ob-

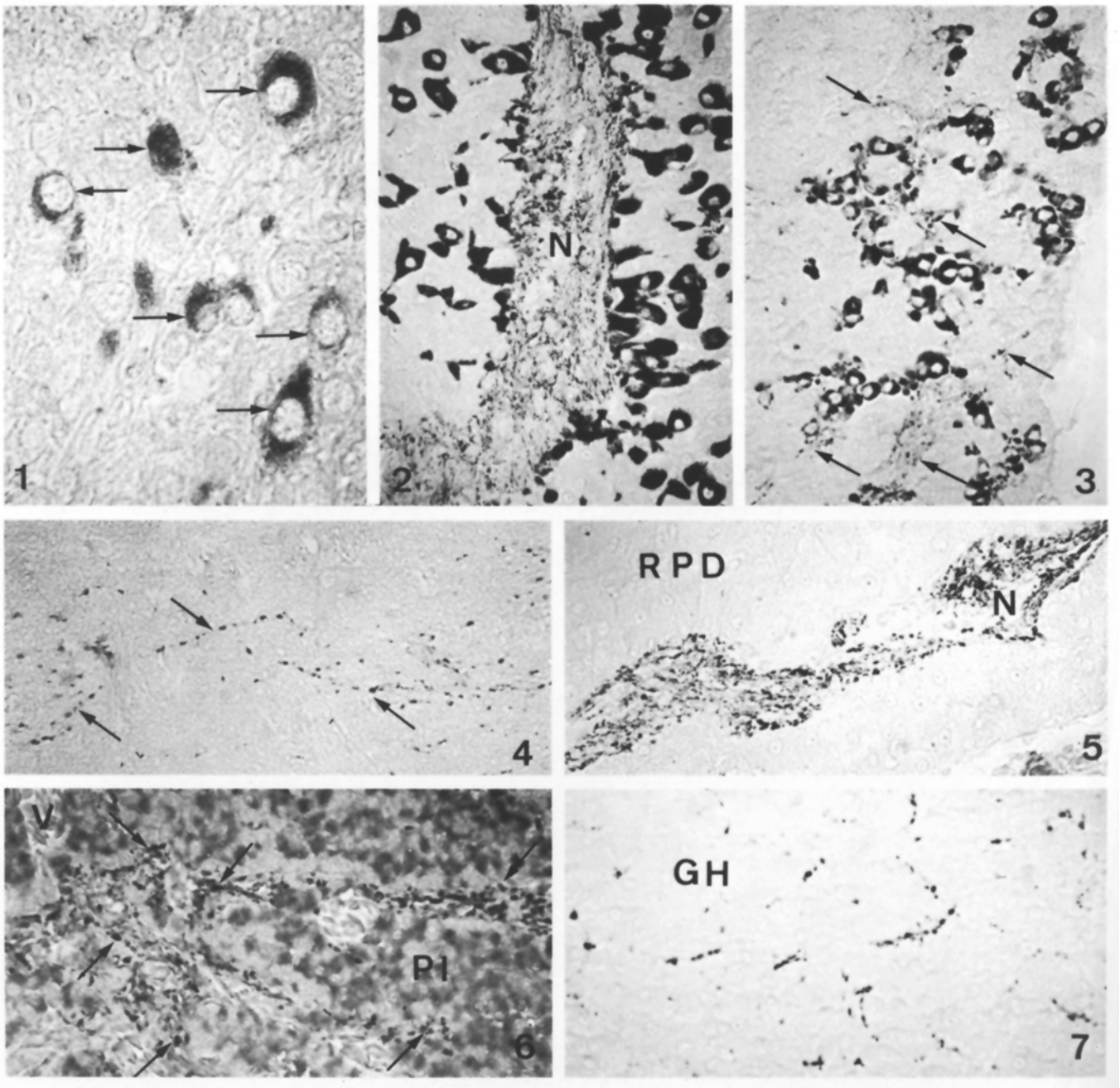

Fig. 1. Sagittal section of goldfish brain showing CRF-like-immunoreactive perikarya scattered in the preoptic nucleus (*arrow*). × 930

Fig. 2. Double staining technique demonstrating numerous CRF-like-immunoreactive fibers in the rostral neurohypophysis (*N*) close to ACTH 1–24-immunoreactive cells in carp pituitary. × 375

Fig. 3. Double staining technique showing fine CRF-like-immunoreactive fibers (*arrows*) in the vicinity of anti-ACTH 1–24-stained cells in the rostral pars distalis of the goldfish. × 375

Fig. 4. Fine CRF-like-immunoreactive fibers in the ventral hypothalamus (*arrows*) of the goldfish. × 440

Fig. 5. Fine CRF-like-immunoreactive fibers in the rostral neurohypophysis (*N*) entering the rostral pars distalis (*RPD*) of the carp. × 375

Fig. 6. Goldfish neurointermediate lobe: ramifications of the caudal neurohypophysis containing CRF-like-immunoreactive fibers with varicosities (*arrows*). In the pars intermedia (*PI*) melanocorticotropic cells are labeled with anti-ACTH 1–24 antiserum; PAS-positive cells are unreactive. × 420

Fig. 7. Varicose SRIF-like-immunoreactive fibers are scattered among GH (growth hormone)-secreting cells in the proximal pars distalis of carp pituitary. × 375

served. Fine fibers run ventrally to the external part of the hypothalamus and form a superficial tract entering the pituitary stalk. These fibers rarely show small varicosities (Fig. 4). Part of the tract turns perpendicularly and penetrates the rostral neurohypophysis (Fig. 5). Most fibers end in front of the ACTH cells. These cells were first identified by their enlargement and degranulation in goldfish treated with aldactone (Olivereau 1964) and metopirone (Fryer and Boudreault-Châteauvert 1981) and by immunocytochemistry (Follénius and Dubois 1976). The double staining illustrates the proximity of ACTH cells and CRF-like-immunoreactive fibers both in the carp (Fig. 2) and the goldfish (Fig. 3). In cyprinids, ACTH cells are mixed with prolactin cells. Staining of consecutive sections with CRF- and isotocin antisera clearly shows that the two nerve tracts do not follow the same pathway.

Some fibers do not seem to enter the pituitary. A few axons may be observed passing dorsally to the third ventricle and extending downward and caudally. In sagittal sections of the brain, transverse sections of immunoreactive fibers are seen ventral to the recessus lateralis and in the anterior periventricular nucleus. Their origin and terminals have not been determined. As the RPD extends ventrolaterally, partly surrounding the PPD, some CRF-like fibers are seen in the lateral areas of the pituitary, occasionally near the GH cells.

The major part of the CRF-like hypothalamic tract runs in a ventro-caudal direction and passes through the PPD. CRF-like-positive fibers making contact with the GH and gonadotropic cells are not observed. The caudal tract ends in the caudal neurohypophysis, among cells of the pars intermedia (PI) (Fig. 6). A basal lamina separates the glandular cells and the nerve fibers in several teleost species. Such a lamina is often lacking or interrupted in the PI of goldfish and carp where PI cells frequently contact nervous tissue. However, in cyprinids typical synaptic contacts between MSH cells and nerve fibers are extremely rare (Kaul and Vollrath 1974; Olivereau et al. 1983). Thick immunoreactive nerve fibers are scattered among MSH cells and the PAS-positive cells. In the goldfish, this last cell type is sensitive to the lack of environmental calcium (Olivereau and Olivereau 1983). Some fibers show large varicosities. The localization of a CRF-like material in cyprinids is similar to that reported in the trout (Bugnon et al. 1983; Ollevier and Verdonck 1984).

The CRF-like-immunoreactive fibers differ from those revealed with an antiserum to SRIF, which are mainly restricted to the PPD. GH cells may be directly innervated and numerous SRIF-like fibers penetrate between GH cells. SRIF-like-immunoreactive fibers are occasionally observed in the caudal neurohypophysis in carps and goldfish. In the pituitary, the SRIF-like fibers appear larger with more frequent varicosities (Fig. 7) compared to the CRF-like fibers. They arise from small perikarya located in the PON, the anterior periventricular nucleus, and the entopeduncular nucleus. The fibers form a dense periventricular plexus. Two preoptico-hypophysial tracts reach the pituitary stalk. Fine fibers may be observed in numerous areas of the brain, even in the olfactory lobes. The present data are in accordance with the localization of a SRIF-like system in larger goldfish (Kah et al. 1982) and some teleost species (Dubois et al. 1979; Vigh-Teichmann et al. 1983; Vigh-Teichmann and Vigh 1983). LHRH, immunoreactive fibers are also restricted to the PPD of goldfish (Kah et al. 1984).

In very few species of teleosts (*Clarias batrachus, Puntius sophore*), a hypothalamo-hypophyseal portal system has been described (Sathyanesan 1972; Sathyanesan and Das 1978). A median eminence similar to that of tetrapods is absent in other species examined so far. Consequently, the presence of a CRF-like neuropeptidergic network in the pituitary of teleost fish must be of functional importance. In the original hypothesis of Diepen (1953), the anterior neurohypophysis, extending from the pituitary stalk to the caudal end of the PPD, is considered as homologous to the median eminence of tetrapods. A similar homology was proposed by Vigh-Teichmann and Vigh (1974). Our immunocytochemical results on CRF-like- and SRIF-like-containing fibers are in accordance with the concept of Diepen.

The stimulation of some parvocellular neurons (PCi) in metopiron-treated goldfish (Fryer and Boudreault-Châteauvert 1981) is concomitant of an increased secretion of ACTH and probably of CRF. This result agrees with the immunocytochemical localization of CRF-like material in similar perikarya.

The CRF-like-immunoreactive fibers seem to end in close proximity to the basal lamina adjacent to the ACTH cells or close to the capillaries bordering the basal lamina. This location suggests that the fibers may control corticotropic activity, either directly when ACTH cells are innervated, or indirectly by releasing a CRF-like peptide into the pericapillary spaces. A direct innervation of ACTH cells seems, however, a very unlikely case in cyprinids (Leatherland 1972; Kaul and Vollrath 1974). Unpublished data collected in other teleost species also support the second hypothesis.

In mammals, CRF enhances release of both α-MSH and ACTH from the anterior and intermediate pituitary (Sakly et al. 1982). The presence of the CRF-like peptide in the NIL suggests that it may also regulate the function of MSH cells in fish as well as in mammals. Indeed, as recently reviewed (Olivereau and Olivereau 1983), MSH cells of fish contain α-MSH, ACTH, and other different neuropeptides. CRF also stimulates secretion of ACTH and β-endorphin in the rat (Vale et al. 1981). In fish, various experimental treatments that stimulate anterior ACTH release activate MSH- or proopiocorticomelanotropic cells (Olivereau 1964). Administration of corticoids inhibiting rostral ACTH release induces a simultaneous hypoactivity of MSH cells (Olivereau 1972). However, MSH cells may react independently of, or more intensely than rostral ACTH cells, for example, after blockade of dopaminergic receptors.

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