Cell Tissue Res (1995) 281:569–572

Cell & Tissue Research

Localization of corticotropin-releasing factor immunoreactivity in the brain of the teleost *Sparus aurata*

Juan Miguel Mancera[1], Pedro Fernández-LLebrez[2]

[1] Departamento de Biología Animal, Facultad de Ciencias del Mar, Universidad de Cádiz, E-11510 Puerto Real, Cádiz, Spain
[2] Departamento de Biología Animal, Facultad de Ciencias, Universidad de Málaga, E-29071 Málaga, Spain

Received: 13 December 1994 / Accepted: 21 March 1995

Abstract. The distribution of perikarya and fibers containing corticotropin-releasing factor (CRF) was studied in the brain of the teleost *Sparus aurata* by immunocytochemistry using the peroxidase-antiperoxidase method. Antisera against rat CRF, arginine vasotocin, and human adrenocorticotropin (ACTH) were used. Most CRF-immunoreactive neurons were located in the nucleus lateralis tuberis, but they were absent from the nucleus preopticus, which only contained arginine vasotocin neurons. Few CRF perikarya were identified in the nucleus preopticus periventricularis and in the mesencephalic tegmentum. A conspicuous bundle of immunoreactive fibers ran along the diencephalic floor and pituitary stalk to end near the cells of the hypophysial pars intermedia. No CRF was seen near the adenohypophysial rostral pars distalis. Our results suggest that, in *Sparus aurata*, CRF is a releasing factor for melanotropic cells. Its role as a releasing factor for ACTH is discussed.

Key words: Corticotropin-releasing factor – Immunocytochemistry – Gilthead sea bream, *Sparus aurata* (Teleostei)

Introduction

Corticotropin-releasing factor (CRF), a 41-amino-acid peptide, was first isolated and characterized from ovine hypothalamus by Vale et al. (1981). This peptide stimulates the release of adrenocorticotropin (ACTH) and melanotropin (MSH) (Meunier et al. 1982; Sakly et al. 1982).

The hypothalamo-infundibular CRF system has been studied immunocytochemically in mammals (Bugnon et al. 1982; Merchenthaler et al. 1982; Kawata et al. 1983; Stolp et al. 1987), birds (Péczeli and Antoni 1984; Bons et al. 1988; Ball et al. 1989), reptiles (Mancera et al. 1991; López Avalos et al. 1993), and amphibians (Tonon et al. 1985; Olivereau et al. 1987). A peptide immunoreactive to CRF antisera has also been found in the brain and hypophysis of teleosts (Bugnon et al. 1983; Olivereau et al. 1984; Yulis et al. 1986; Yulis and Lederis 1987; Olivereau and Olivereau 1988), ganoids (Belenky et al. 1985), and elasmobranchs (Vallarino et al. 1989). In general, CRF neurons have been localized in the preoptic region in fish; these neurons project axons to the neurohypophysis near ACTH and MSH cells. Since teleosts represent a diverse group of vertebrates, and the distributional patterns of their central nervous peptide systems display considerable variation, we have studied the CRF system of *Sparus aurata*.

Correspondence to: P. Fernández-LLebrez

Materials and methods

Seven juvenile gilthead sea bream *Sparus aurata* (about 100–150 g body weight) were used. The specimens were obtained from sea water (980 mOsm/kg) and were kindly supplied by the fish culturing center El Toruño (PEMARES), El Puerto de Santa María, Cádiz, Spain. Fish were anesthetized with aminobenzoic acid (MS 222; Sigma Madrid) dissolved in the ambient water (0.065 g/l) and killed by decapitation. The brains were dissected and placed in Bouin's fluid for 48 h; they were then dehydrated and embedded in paraffin. Sagittal and transverse sections (8 µm thick) were immunostained according to the unlabeled enzyme method of Sternberger (1986) using the following primary antisera: anti-rat CRF (1:500) from E.M. Rodríguez, Valdivia, Chile, anti-human ACTH (1:1500) from Peninsula Laboratory, Calif., and anti-arginine vasotocin(AVT) from R.M. Buijs, Amsterdam, Holland. All sections were incubated for 18 h at 22° C in the primary antisera, and for 30 min at 22° C in the second antiserum (anti-rabbit IgG developed in goat, 1:10), and in the PAP complex (1:75) (Sigma). DAB was used to reveal the peroxidase. All antisera and the PAP complex were diluted in 0.05 M TRIS buffer, pH 7.8, containing 0.7% non-gelling seaweed gelatin, lambda carrageenan (Sigma), 0.5% Triton X-100 (Sigma), and 0.02% sodium azide. Coplin jars were used for incubation in the first and the second antisera, whereas the PAP incubation was carried out in a moist chamber. The specificity of the anti-rat CRF was tested by immunoabsorption with rat CRF (Mancera et al. 1991). Immunoabsorbed anti-CRF stained no structures in sections of the sea bream brain. Anti-AVT serum was previously tested by Fernández-LLebrez et al. (1988). In order to control the immunoreactive procedure, contig-

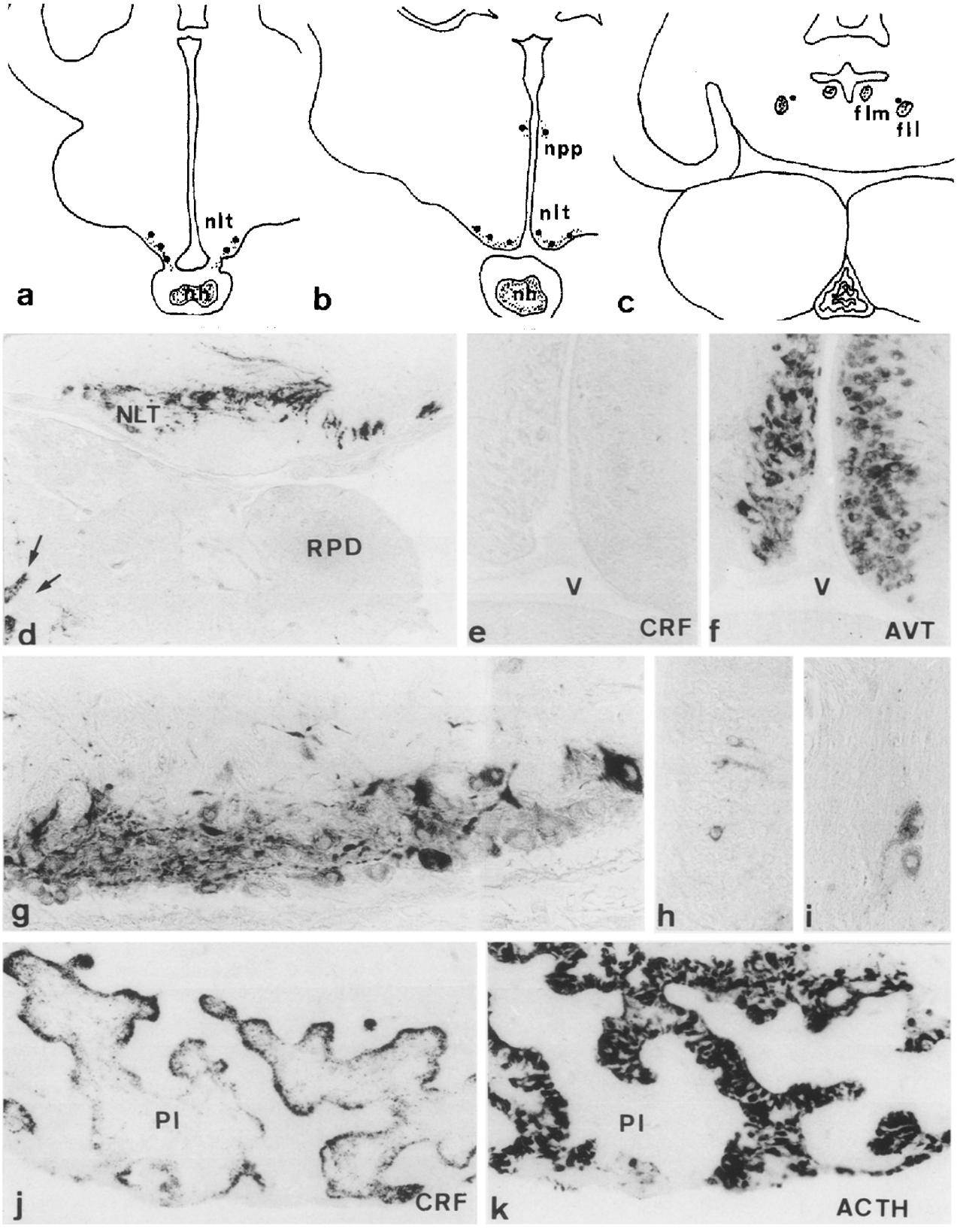

Fig. 1. a–c Schematic drawings of transverse sections through the brain of *S. aurata* showing the localization of CRF-imunoreactive perikarya (*dark circles*) and fibers (*dots*). *fll* Fasciculus longitudinalis lateralis; *flm* fasciculus longitudinalis medialis; *nh* neurohypophysis; *nlt* nucleus lateralis tuberis; *npp* nucleus preopticus periventricularis. **d** Sagittal section through the diencephalic floor and hypophysis. CRF cells and fibers are abundant in the nucleus lateralis tuberis (*NLT*). In the neurohypophysis, CRF fibers lie near the pars intermedia (*arrows*). The rostral pars distalis (*RPD*) is devoid of immunoreactive fibers. ×135. **e, f** Adjacent transverse sections through the nucleus preopticus immunostained with antisera against CRF and AVT, respectively. *V* Third ventricle. ×125. **g** Sagittal section of the nucleus lateralis tuberis showing many CRF-immunoreactive cells and fibers. ×280. **h** CRF-immunoreactive perikarya in the nucleus preopticus periventricularis. ×430. **i** CRF-immunoreactive neurons in the mesencephalic tegmentum. ×300. **j, k** Adjacent transverse sections through the neurointermediate lobe of the hypophysis immunostained with antisera against CRF and ACTH, respectively. CRF fibers end near the cells of the pars intermedia (*PI*). ×135

uous sections were immunostained, omitting the incubation in the primary antisera. No positive structures were found in control sections.

Results and discussion

The distribution of CRF-immunoreactive (-IR) perikarya and fibers in transverse sections of the brain of *S. aurata* is presented schematically in Fig. 1a–c. A conspicuous group of CRF-IR perikarya is present in the nucleus lateralis tuberis (NLT) (Fig. 1d, g). No immunoreactivity is however seen in the nucleus preopticus (NPO) (Fig. 1e), where most neurons appear to be immunoreactive to the anti-AVT serum (Fig. 1f). The CRF-IR neurons are large and display round, fusiform or pear-shaped perikarya. In addition a few, small, weakly immunoreactive neurons can be seen in the nucleus preopticus periventricularis (NPP) (Fig. 1h) and in the mesencephalic tegmentum, in an area between the fasciculi longitudinales medialis and lateralis (Fig. 1i).

A conspicuous bundle of CRF-IR fibers is present along the hypothalamic floor, the pituitary stalk, and the neural region of the neurointermediate hypophysial lobe. Here, nerve endings outline the palisade region close to the cells of the intermediate lobe that stain with the anti-human ACTH serum (Fig. 1j, k). No CRF-IR fibers have been found in the rostral pars distalis of the adenohypophysis (RPD) (Fig. 1d).

The anti-rat CRF serum strongly stains cells and fibers in the brain of *S. aurata*. Since immunoreactivity is abolished by preincubation of the antiserum with CRF, it is likely that, as in other teleost species, a CRF-like peptide exists in the brain of *S. aurata*.

CRF-IR neurons have previously been reported in the NPO and NLT of teleost fishes (Bugnon et al. 1983; Olivereau et al. 1984; Yulis et al. 1986; Yulis and Lederis 1987; Olivereau and Olivereau 1988). Unlike all other teleosts studied, CRF is absent from the NPO in *S. aurata*; thus, the NLT is the only CRF neuronal group projecting to the pituitary.

In other teleost species, the NPO has been reported to be the main source of CRF fibers innervating the hypophysial RPD, where they presumably control the release of ACTH (Bugnon et al. 1983; Olivereau et al. 1984; Olivereau and Olivereau 1988). However, in *Catostomus commersoni*, the fibers innervating RPD arise from the NLT, whereas the NPO innervates the neurointermediate lobe (Yulis and Lederis 1987). In *S. aurata*, the CRF cells of the NLT seem to innervate the neurointermediate lobe, whereas the RPD receives no CRF fibers. Since the main population of ACTH cells in the adenohypophysis of the sea bream is located in the RPD (Quesada et al. 1988), the following possibilities can be considered: (1) CRF is not a releasing factor for ACTH in *S. aurata* or (2) CRF reaches the RPD via blood vessels or in a paracrine fashion. Another possibility is that the intermediate lobe cells of *S. aurata* contain ACTH in addition to MSH.

In mammals, CRF stimulates the release of ACTH, β-endorphin, and MSH (Vale et al. 1981; Meunier et al. 1982; Sakly et al. 1982). In fishes, morphological studies have revealed a coordinated response of ACTH and MSH cells (Olivereau 1972) and physiological studies have suggested that CRF controls the release of MSH (Lederis 1987). We have reported that, in *S. aurata*, adaptation to brackish water induces hyperactivity of ACTH cells and increased levels of plasma cortisol together with the stimulation of MSH cells (Mancera et al. 1993a, b). These observations together with the present results indicate that, as for mammals and other teleosts (Holmes and Ball 1974), CRF may be a releasing factor for MSH cells of the intermediate lobe in *S. aurata*.

The presence of CRF-IR cells in the NPP has also been reported in other teleosts (Olivereau and Olivereau 1988). Its occurrence in extrahypothalamic regions suggests that it could act as a central neurotransmiter.

Acknowledgements. This work was supported by Grants GRPRE 93-08 Universidad de Cádiz, Grupo PAI 5403, Junta de Andalucía to J.M.M., and DGICYT PB93-0979 Madrid to P.F.L.L.

References

Ball GF, Faris PL, Wingfield JC (1989) Immunohistochemical localization of corticotropin-releasing factor in selected brain areas of the European starling (*Sturnus vulgaris*) and the song sparrow (*Melospiza melodia*). Cell Tissue Res 257:155–161

Belenky MA, Kuzik VV, Chernigovskaya EV, Polenov AL (1985) The hypothalamo-hypophysial system in Acipenseridae. X. Corticoliberin-like immunoreactivity in the hypothalamus and hypophysis of *Acipenser ruthenus* L. Gen Comp Endocrinol 60:20–26

Bons N, Bouille C, Tonon MC, Guillaume V (1988) Topographical distribution of CRF immunoreactivity in the pigeon brain. Peptides 9:697–707

Bugnon C, Fellman D, Bresson JL, Clavequin MC (1982) Etude immunocytochimique de l'ontogénèse du système neuroglandulaire a CRF chez l'homme. C R Acad Sci III 294:491–496

Bugnon C, Cardot J, Gouget A, Fellmann D (1983) Mise en évidence d'un système neuronal peptidergique réactif à un immunserum anti-CRF41, chez les téléostéens dulcicoles et marins. C R Acad Sci III 296:711–716

Fernández-LLebrez P, Pérez J, Nadales AE, Cifuentes M, Grondona JM, Mancera JM, Rodríguez EM (1988) Immunocytochemical study of the hypothalamic magnocellular neurosecretory nuclei of the snake *Natrix maura* and the turtle *Mauremys caspica*. Cell Tissue Res 253:435–445

Holmes RL, Ball JN (1974) The pituitary gland: a comparative account. Cambridge University Press, London, New York

Kawata M, Hashimoto K, Takahara J, Sano Y (1983) Immunohistochemical identification of neurons containing corticotropin-releasing factor in the rat hypothalamus. Cell Tissue Res 230:239–246

Lederis K (1987) Non-mammalian corticotropin release-stimulating peptides. Ann NY Acad Sci 512:129–138

López Avalos MD, Mancera JM, Pérez-Fígares JM, Fernández-LLebrez P (1993) Immunohistochemical localization of corticotropin-releasing factor in the brain of the turtle *Mauremys caspica*. Cell Tissue Res 188:163–171

Mancera JM, López Avalos MD, Pérez-Fígares JM, Fernández-LLebrez P (1991) The distribution of corticotropin-releasing factor-immunoreactive neurons and fibers in the brain of the snake *Natrix maura*. Cell Tissue Res 264:539–548

Mancera JM, Fernández-LLebrez P, Grondona JM, Pérez-Fígares JM (1993a) Influence of environmental salinity on prolactin and corticotropic cells in the gilthead sea bream (*Sparus aurata* L.). Gen Comp Endocrinol 90:220–231

Mancera JM, Fernández-Llebrez P, Pérez-Fígares JM (1993b) Efecto de la salinidad ambiental sobre las células melanotrópicas de la dorada (*Sparus aurata* L.). Rev Esp Fisiol 49:115–120

Merchenthaler I, Vigh S, Petrusz P, Schally AV (1982) Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain. Am J Anat 165:385–396

Meunier H, Lefévre G, Dumont D, Labrie F (1982) CRF stimulates alpha-MSH secretion and cyclic AMP accumulation in pars intermedia cells. Life Sci 31:2129–2136

Olivereau M (1972) Actions d'un apport de cortisol sur la cytologie de l'hypophyse chez l'Anguille. Acta Zool 53:179–194

Olivereau M, Olivereau J (1988) Localization of CRF-like immunoreactivity in the brain and pituitary of teleost fish. Peptides 9:13–21

Olivereau M, Ollevier F, Vandesande F, Verdonck W (1984) Immunocytochemical identification of CRF-like and SRIF-like peptides in the brain and the pituitary of cyprinid fish. Cell Tissue Res 237:379–382

Olivereau M, Vandesande F, Boucique E, Ollevier F, Olivereau JM (1987) Immunocytochemical localization and spatial relation to the adenohypophysis of a somatostatin-like and a corticotropin-releasing factor-like peptide in the brain of four amphibian species. Cell Tissue Res 247:317–324

Péczely P, Antoni FA (1984) Comparative localization of neurons containing ovine corticotropin releasing factor (CRF)-like and neurophysin-like immunoreactivity in the diencephalon of the pigeon (*Columba livia domestica*). J Comp Neurol 228:69–80

Quesada J, Lozano Mt, Ortega A, Agulleiro B (1988) Immunocytochemical and ultrastructural characterization of the cell types in the adenohypophysis of *Sparus aurata* L. (Teleost). Gen Comp Endocrinol 72:209–225

Sakly M, Schmitt G, Koch B (1982) CRF enhances release of both alpha-MSH and ACTH from the anterior and intermediate pituitary. Neuroendocrinol Lett 4:289–293

Sternberger LA (1986) Immunocytochemistry. 3rd edn. Wiley, New York Chichester Brisbane

Stolp R, Steinbuch HWM, Rijnberk A, Croughs RJM (1987) Organization of ovine corticotropin-releasing factor immunoreactive neurons in the canine hypothalamo-pituitary system. Neurosci Lett 74:337–342

Tonon MC, Burlet A, Lauber M, Cuet P, Jegou S, Gouteux L, Ling N, Vaudry H (1985) Immunohistochemical localization and radioimmunoassay of corticotropin-releasing factor in the forebrain and hypophysis of the frog *Rana ridibunda*. Neuroendocrinology 40:109–119

Vale W, Spiess J, Rivier C, Rivier J (1981) Characterization of a 41-residue bovine hypothalamic peptide that stimulates secretion of corticotropin and β-endorphin. Science 213:1394–1397

Vallarino M, Fasolo A, Ottonello I, Perroteau L, Tonon TC, Vandesande F, Vaudry H (1989) 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

Yulis CR, Lederis K (1987) Colocalization of the immunoreactivities of corticotropin-releasing factor and arginine vasotocin in the brain and pituitary system of the *Catostomus commersoni*. Cell Tissue Res 247:267–273

Yulis CR, Lederis K, Wong KL, Fisher AW (1986) Localization of urotensin I- and corticotropin-releasing factor-like immunoreactivity in the central nervous system of *Catostomus commersoni*. Peptides 7:79–86