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Distribution of gonadotropin-releasing hormone immunoreactive systems in the brain of the Senegalese sole, *Solea senegalensis*

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Summary

The present paper reports the immunohistochemical distribution of the gonadotropin-releasing hormone (GnRH) structures in the brain of the Senegalese sole, *Solea senegalensis*. In this study, we have used two antibodies against the salmon GnRH and chicken GnRH-II forms and the streptavidin–biotin-peroxidase complex method. Immunoreactive cell bodies are observed at the junction between the olfactory bulbs and the telencephalon (terminal nerve ganglion cells), in the ventral telencephalon, in the preoptic parvocellular nucleus, and in the synencephalic nucleus of the medial longitudinal fasciculus. GnRH-immunoreactive fibres were found extensively throughout the brain, located in the telencephalon, preoptic area, hypothalamus, hypophysis, optic tectum, midbrain and rhombencephalon. The antisera used in this study against the two GnRH forms exhibited cross-reactivity on the same cell masses and did not allow cell populations expressing different GnRH forms to be discriminated clearly. However, anti-salmon GnRH immunostained the GnRH cells and fibres of the forebrain much more intensely, whereas the anti-chicken GnRH antiserum shows a higher immunoreactivity on synencephalic cells of the medial longitudinal fasciculus.

Introduction

It has been clearly established that the brain, and especially the forebrain, plays an important role in the control of the reproductive process (Kah *et al*. 1993). Gonadotropin-releasing hormone (GnRH) represents the main cerebral factor responsible for the secretion of gonadotropins from the pituitary (Breton *et al*. 1972, King & Millar 1992, Sherwood *et al*. 1993). In the last twenty-eight years, a multiplicity of highly conserved GnRH variants have been described throughout the vertebrate phylogeny. At present, ten structurally different forms of GnRH have been characterized from vertebrate brains and two additional forms have been isolated in a protochordate: mammalian GnRH (mGnRH; Matsuo *et al*. 1971, Burgus *et al*. 1972), chicken GnRH-I (cGnRH-I; King & Millar 1982a, Miyamoto *et al*. 1982), chicken GnRH-II (cGnRH-II; King & Millar 1982b, Miyamoto *et al*. 1984), salmon GnRH (sGnRH; Sherwood *et al*. 1983), lamprey GnRH-I (lGnRH-I; Sherwood *et al*. 1986), lamprey GnRH-III (lGnRH-III; Sower *et al*. 1993), catfish GnRH (cfGnRH; Ngamvongchon *et al*. 1992), dogfish GnRH (dfGnRH; Lovejoy *et al*. 1992), seabream GnRH (sbGnRH; Powell *et al*. 1994), guinea pig GnRH (gpGnRH, Jiménez-Liñan *et al*. 1997), tunicate-1 and tunicate-2 GnRH (tun1GnRH and tun2GnRH, Powell *et al*. 1996).

The presence of at least two GnRH variants has been extensively referred in the brain of non-mammalian vertebrates species (Sherwood *et al*. 1984, Sherwood 1986, Yu *et al*. 1988, Okuzawa *et al*. 1990, King & Millar 1992, Muske 1993, Kah *et al*. 1993, Yamamoto *et al*. 1995, Goos *et al*. 1997). The basic pattern of distribution of GnRH cells was established in some teleosts via immunohistochemistry and suggested the existence of two major GnRH systems: one system along the ventral forebrain (terminal nerve, ventral telencephalon and preoptic area) expressing either sGnRH, mGnRH, cfGnRH or sbGnRH, and another system in the midbrain tegmentum expressing cGnRH-II (Münz *et al*. 1981, Goos *et al*. 1985, Kah *et al*. 1986, Kah *et al*. 1991, Subheader & Rama Krishna 1988, Batten *et al*. 1990, Amano *et al*. 1991, Grober & Bass 1991, Yamamoto *et al*. 1995). However, recent data have demonstrated, at least in perciform, that three GnRH forms are expressed in three different brain regions: sGnRH in the olfactory bulb, sbGnRH in the preoptic region, and cGnRH-II in the dorsal tegmentum (Powell *et al*. 1994, White *et al*. 1995, Gothilf *et al*. 1996, Okuzawa *et al*. 1997).

The Senegalese sole, *Solea senegalensis*, is a pleuronectiform fish characteristic of Atlantic and Mediterranean coasts, which is also exploited in extensive and intensive aquaculture in some southern European countries, such as Spain (Drake *et al*. 1984) and Portugal (Dinis 1992), and on the African coasts of Tunisia (Fehri-Bedoui 1997). Thus, the complete control of sexual maturation and spawning of this species represents an important objective for fish farmers. The success of this activity is strongly dependent on the understanding of

the mechanisms involved in the hormonal control of spawning under culture conditions, in which GnRH plays a major role. Recently, immunohistochemical, histochemical and/or biochemical studies of oogenesis (Gutiérrez *et al*. 1985, Sarasquete *et al*. 1993), larval development (Mourente & Vázquez 1996, Sarasquete *et al*. 1996), and pituitary cells (Rendón *et al*. 1997) have been addressed in this species. However, no attention has been paid to elucidate the distribution of GnRH and other neuroendocrine factors implicated in the control of the reproductive process. This fact is in part the consequence of the lack of specific neuroanatomical information for this kind of study. In this paper we examine the distribution of immunoreactive-GnRH cells and fibres in the brain of the Senegalese sole, *Solea senegalensis* with the help of a brain atlas recently developed in our laboratory (F.J. Rodríguez-Gómez, C. Sarasquete & J.A. Muñoz-Cueto, in preparation). The major aim of this study is to obtain preliminary information on the neuroendocrine control of the gonadotropic function of this commercially important species.

Materials and methods

Adult specimens of Senegalese sole, *Solea senegalensis* ($n = 17$), were purchased from a local fishery (Cupimar, S.A. San Fernando, Spain) and kept in the laboratory in running seawater. Animals were anaesthetized with 2-phenoxyethanol (Sigma, St. Louis) and perfused via the aortic bulb with 0.6% saline solution, followed by Bouin's fixative (4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, 0.2% picric acid). Brains with the pituitary attached were then carefully removed and further postfixed in the same fixative for 6 h in the dark at 4 °C.

After fixation, brains were cryoprotected overnight in 0.1 M phosphate buffer containing 15% sucrose, embedded in Tissue-Tek, frozen in cold isopentane and kept at −80 °C until processed. Serial transversal sections 16 μm-thick, were cut in a cryomicrotome and mounted on gelatin-coated glass-slides.

Immunohistochemical staining was performed using a streptavidin–biotin-peroxidase complex method. Endogenous peroxidase activity was blocked with 1% hydrogen peroxide in Coons buffer (CBT; 0.01 M Veronal, 0.15 M NaCl 0.1% Triton X-100) for 30 min. Before immunostaining, sections were transferred for 5 min to CBT and then to CBT containing 0.5% casein for 30 min. Consecutive serial sections were incubated overnight in a moist chamber at room temperature with anti-salmon GnRH (donated by Dr. Breton) and anti-chicken GnRH-II (donated by Dr. Peute) antisera diluted 1 : 2000 in CBT containing 0.5% casein. The specificity of the antisera used in this study have been confirmed previously (Breton *et al*. 1986, Schulz *et al*. 1993, Kah *et al*. 1994). Sections were washed in CBT (3 × 10 min) and incubated for 2 h at room temperature with biotinylated anti-rabbit-IgG diluted 1 : 1000 in CBT. After washing in CBT (3 × 5 min), sections were incubated for 2 h at room temperature with streptavidin-peroxidase complex diluted 1 : 1000 in CBT. Finally, sections were washed in CBT followed by Tris-HCl (0.05 M, pH 7.4) and peroxidase activity visualized with 0.05 M Tris-HCl, pH 7.6, containing 0.025% 3,3′-diaminobenzidine tetrahydrochloride (Sigma, St Louis, MO) and 0.01% hydrogen peroxide or 0.04% 4-chloro-1-naphthol (Sigma, St Louis, MO) and 0.01% hydrogen peroxide. To confirm the specificity of the immunostaining, controls were performed by the replacement of primary antisera with rabbit normal serum and omission of primary and biotinylated antisera. The sections were mounted in an aqueous mounting medium for microscopy (Aquatex, Merck), observed on a Leitz photomicroscope and photographed using panchromatic Agfapan APX 25 Films.

The precise localization of GnRH cells and fibres was determined with the help of a sole brain atlas previously elaborated in our laboratory (F.J. Rodríguez-Gómez, C. Sarasquete & J.A. Muñoz-Cueto, in preparation).

Results

Transverse serial sections of Senegalese sole brains were processed according to a streptavidin–biotin-peroxidase complex immunohistochemical method, using anti-salmon GnRH and anti-chicken GnRH-II as primary antisera. Representative brain sections of the Senegalese sole showing immunoreactive fibers and cell bodies are presented in Figure 1.

In the Senegalese sole, antiserum against sGnRH intensely immunostains a group of large cell bodies that lie just rostral to the junction of the olfactory bulbs with the ventral telencephalon (Figures 1A, 2A). These GnRH-positive neurons correspond to the ganglion cells of the terminal nerve. In the telencephalon, sGnRH-immunoreactive cells are observed in the area ventralis pars ventralis (Figures 1B, 2B and 2C). In the diencephalon, a sGnRH cell population is observed in the nucleus preopticus parvocellularis pars parvocellularis of the preoptic area (Figures 1C, 2D). The antiserum against cGnRH-II exhibits an intense immunoreactivity in a cell population of the synencephalon, a region placed between the diencephalon and the mesencephalon. These large cells are located in the medial part of the nucleus of the medial longitudinal fasciculus (Figures 1F, 2E). However, less intensely stained cGnRH-II-immunoreactive cell bodies are also observed in the anterior ventral regions, where sGnRH-immunoreactive perikarya are detected, and conversely, weaker sGnRH immunoreactivity is observed in the medial longitudinal fasciculus nucleus where cGnRH-II-positive cell bodies are found.

The sGnRH-immunoreactive fibres are widely distributed in the Senegalese sole brain. These fibres are more evident along the ventral surface of the brain, extending from the olfactory bulbs (Figures 1A, 2A) to the hypophysis (Figures 1D, 3A) via the ventral telencephalon (Figures 1B, 2B, C), preoptic area (Figures 1C, 2D) and mediobasal hypothalamus (Figures 1D, E, 3A). Furthermore, GnRH-immunoreactive fibres can be observed in the

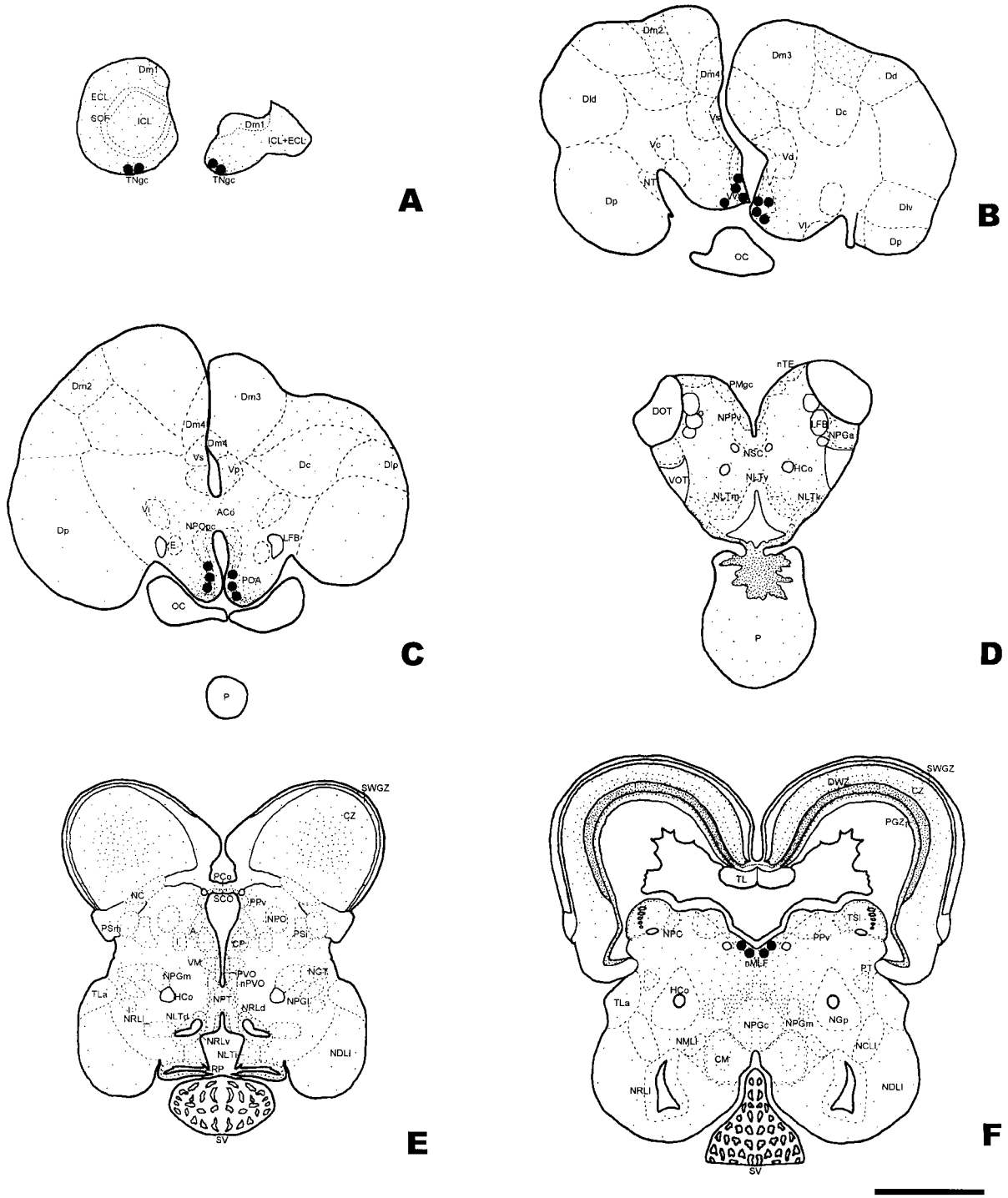

Figure 1. Series of transverse sections throughout the brain of *Solea senegalensis* showing the distribution of GnRH cells (large filled circles) and fibres (small dots). In this figure, no attempt was made to distinguish sGnRH from cGnRH-II given the possible cross-reactivity of anti-sGnRH and anti-cGnRH-II antisera on the same cell masses. For more details see the text. A, constitutes the rostralmost section and J, the caudalmost one. Bar scale = 1 mm. For abbreviations, see Table 1.

dorsomedial (mainly in Dm2), dorsocentral, dorsolateral and dorsal-posterior telencephalon (Figure 1B, C), in the medial thalamus (Figure 1E) and hypothalamus (Figure 1E, F), pretectal area (Figure 1E), the deep white zone and central zone of the optic tectum (Figures 1E–H, 3B) and the torus semicircularis (Figure 1F–H). In the caudal brain, scattered immunoreactive fibres are observed in the valvula and corpus cerebelli (mainly in the granular layer and around the Purkinje cells), vagal lobe, octavolateral area, reticular formation and ventral rhombencephalon (Figure 1G–J). The distribution of cGnRH-II fibres is basically similar to that of sGnRH-immunoreactive fibres but the numbers of reactive fibres were slightly fewer than those of sGnRH, especially in the forebrain.

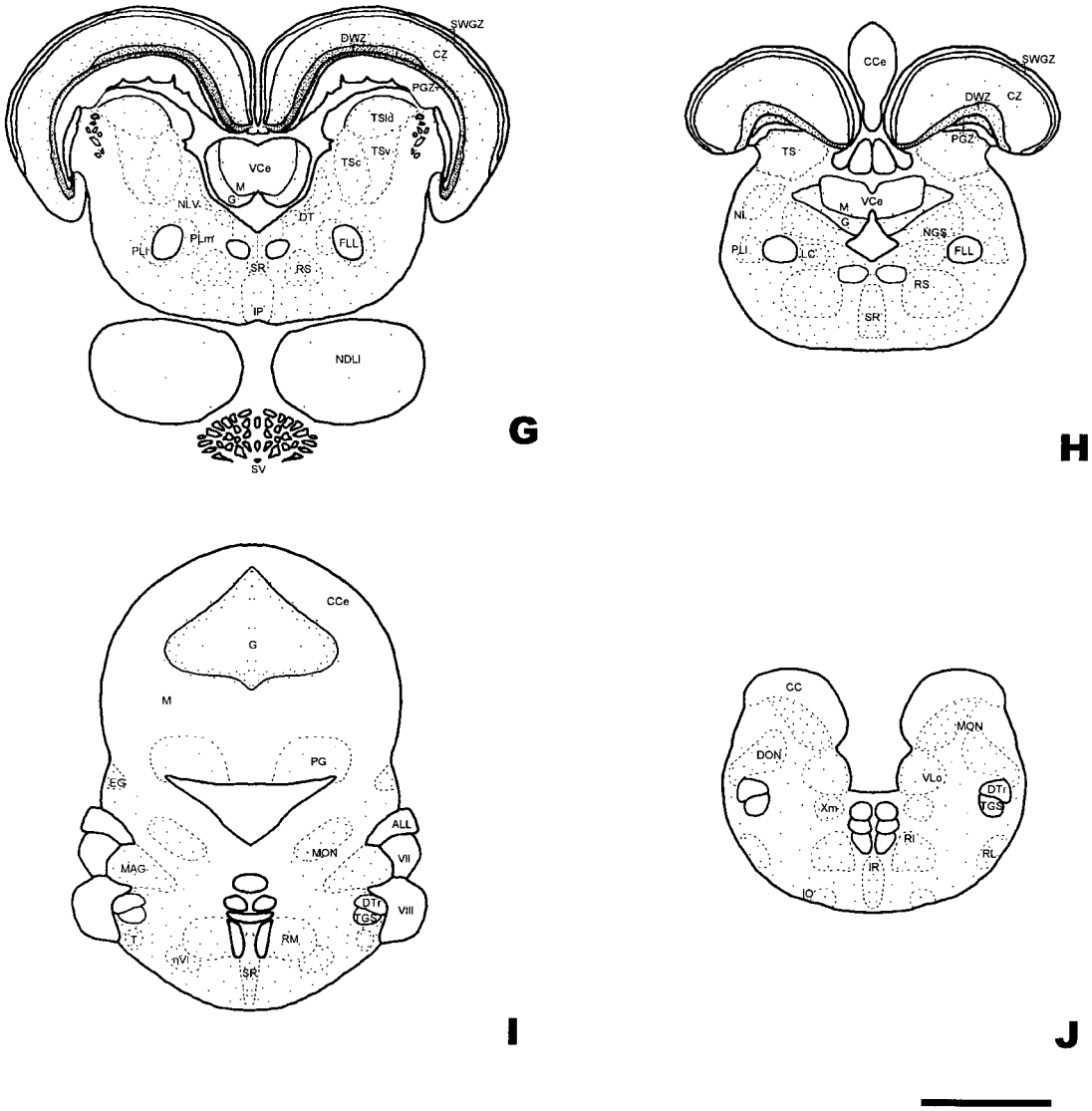

Figure 1. (*Continued*)

Discussion

The present study reveals the distribution of GnRH-immunoreactive cells and fibres in the brain of a pleuronectiform fish, *Solea senegalensis*, using antisera against two different types of GnRH, i.e. sGnRH and cGnRH-II. The antiserum against sGnRH immunostains intensely three forebrain cell masses: terminal nerve ganglion cells, ventral telencephalic cells and parvocellular preoptic cells, whereas the antiserum against cGnRH-II shows an intense immunoreactivity in synencephalic cells of the nucleus of the medial longitudinal fasciculus. This study demonstrated that the overall central distribution of the GnRH systems in Senegalese sole is highly similar to what has been described in other marine and freshwater teleosts (Münz *et al*. 1981, Goos *et al*. 1985, Kah *et al*. 1986, Subheader & Rama Krishna 1988, Batten *et al*. 1990, Kah *et al*. 1991, Amano *et al*. 1991, Grober & Bass 1991, Montero *et al*. 1994, Yamamoto *et al*. 1995, Parhar 1997). However, in another member of the Soleidae family, *Solea solea*, only terminal nerve and preoptic GnRH centres were detected (Nunez-Rodríguez *et al*. 1985). An additional GnRH cell population has been described in the mediobasal hypothalamus of goldfish (Kah *et al*. 1986), sea bass (Kah *et al*. 1991), sturgeon (Leprêtre *et al*. 1993), European eel (Montero *et al*. 1994) or rainbow trout (Navas *et al*. 1995), which has not been observed in Senegalese sole and other teleosts (Oka & Ichikawa 1990, Amano *et al*. 1991, Coe *et al*. 1992).

In the Senegalese sole, immunostained cGnRH-II cell bodies are also observed in the ventral forebrain, whereas sGnRH immunoreactivity is also detected in the most caudal GnRH perikarya of the synencephalon. Nevertheless, midbrain GnRH cells were not detected immunocytochemically in masu salmon (Amano *et al*. 1991) or sea bass (Kah *et al*. 1991) using anti-sGnRH antibodies. Both forebrain and synencephalic GnRH cell masses were also evident in the platyfish (Münz *et al*. 1981) and the goldfish (Kah *et al*. 1986) using antisera against mGnRH and sGnRH,

Table 1. Abbreviations of neuroanatomical regions of the Senegalese sole brain.

A: nucleus anterior thalami
ACo: anterior commissure
ALL: anterior lateral line nerve
CC: crista cerebellaris
CCe: corpus cerebelli
CM: corpus mammillare
CP: nucleus centralis posterior thalami
CZ: central zone
Dc: area dorsalis telencephali pars centralis
Dd: area dorsalis telencephali pars dorsalis
Dld: area dorsalis telencephali pars lateralis dorsal
Dlp: area dorsalis telencephali pars lateralis posterior
Dlv: area dorsalis telencephali pars lateralis ventral
Dm1: area dorsalis telencephali pars medialis subdivision 1
Dm2: area dorsalis telencephali pars medialis subdivision 2
Dm3: area dorsalis telencephali pars medialis subdivision 3
Dm4: area dorsalis telencephali pars medialis subdivision 4
DON: nucleus octavus descendens
DOT: dorsal optic tract
Dp: area dorsalis telencephali pars posterioris
DT: nucleus tegmentalis dorsalis
DTr: descending trigeminal tract
E: nucleus entopeduncularis
ECL: external cellular layer
EG: eminentia granularis
FLL: fasciculus longitudinalis lateralis
G: granular layer of the cerebellum
GL: glomerular layer
HCo: horizontal commissure
I: nucleus intermedius thalami
ICL: internal cellular layer
IO: inferior olive
IP: nucleus interpeduncularis
IR: nucleus raphes inferior
LC: nucleus of the locus coeruleus
LFB: lateral forebrain
M: molecular layer of the cerebellum
MAG: nucleus magnocellularis
MON: nucleus octavolateralis medialis
NC: nucleus corticalis
NCLI: nucleus centralis lobi inferioris
NDLI: nucleus diffusus lobi inferioris
NGp: nucleus glomerulosus pars posterioris
NGS: nucleus gustatorius secundarius
NGT: nucleus gustatorius tertius
NH: neurohypophysis
NI: nucleus ithsmi
NLT: nucleus lateralis tuberis
NLTd: nucleus lateralis tuberis pars dorsalis
NLTi: nucleus lateralis tuberis pars inferioris
NLTlr: nucleus lateralis tuberis pars lateralis rostralis
NLTm: nucleus lateralis tuberis pars medialis
NLTv: nucleus lateralis tuberis pars ventralis
NLV: nucleus lateralis valvulae
nMLF: nucleus of the medial longitudinal fasciculus
NMLI: nucleus medialis lobi inferioris
NPC: nucleus pretectalis centralis
NPGa: nucleus preglomerulosus anterioris
NPGc: nucleus preglomerulosus commissuralis
NPGl: nucleus preglomerulosis lateralis
NPGm: nucleus preglomerulosus medialis
NPOpc: nucleus preopticus parvocellularis pars parvocellularis
NPPv: nucleus posterioris periventricularis
NPT: nucleus posterior tuberis
nPVO: nucleus of the paraventricular organ
NRLd: nucleus recessus lateralis pars dorsalis
NRLv: nucleus recessus lateralis pars ventralis
NRLl: nucleus recessus lateralis pars lateralis
NSC: nucleus suprachiasmaticus
NT: nucleus taenia
nTE: nucleus eminentia thalami
nVI: nucleus nervi abducentis
OB: olfactory bulbs
OC: optic chiasm
OlN: olfactory nerve fibres
P: pituitary
PCo: posterior commissure
PG: periventricular granular cell mass
pgd: nucleus periglomerulosus dorsalis
PGZ: periventricular grey zone
PLl: nucleus perilemniscularis pars lateralis
PLL: posterior lateral line nerve
PLm: nucleus perilemniscularis pars medialis
PMgc: nucleus preopticus magnocellularis pars gigantocellularis
POA: preoptic area
PPv: nucleus pretectalis periventricularis pars ventralis
PSi: nucleus pretectalis superficialis pars intermedius
PSm: nucleus pretectalis superficialis pars magnocellularis
PT: nucleus posterior thalami
PVO: paraventricular organ
RI: nucleus reticularis inferioris
RL: nucleus reticularis lateralis
RP: recessus posterioris
rpo: recessus preopticus
RS: nucleus reticularis superioris
SCO: subcommissural organ
SOF: secondary olfactory fibres
SR: nucleus raphes superior
SV: saccus vasculosus
SWGZ: superficial white and grey zone
T: nucleus tangentialis
TGS: tractus gustatorius secundarius
TL: torus longitudinalis
TLa: nucleus tori lateralis
TNgc: terminal nerve ganglionar cells
TS: torus semicircularis
TSc: torus semicircularis pars centralis
TSl: torus semicircularis pars lateralis
TSld: torus semicircularis pars lateralis dorsalis
TSlv: torus semicircularis pars lateralis ventralis
TSv: torus semicircularis pars ventralis
Vc: area ventralis telencephali pars centralis
VCe: valvula cerebelli
Vd: area ventralis telencephali pars dorsalis
Vi: area ventralis telencephali pars intermedia
VII: nervus facialis
VIII: nervus octavus
Vl: area ventralis telencephali pars lateralis
VLo: vagal lobe
VM: nucleus ventromedialis thalami
VOT: ventral optic tract
Vp: area ventralis telencephali pars postcommissuralis
Vs: area ventralis telencephali pars supracommissuralis
Vv: area ventralis telencephali pars ventralis
Xm: nucleus motorius nervi vagi

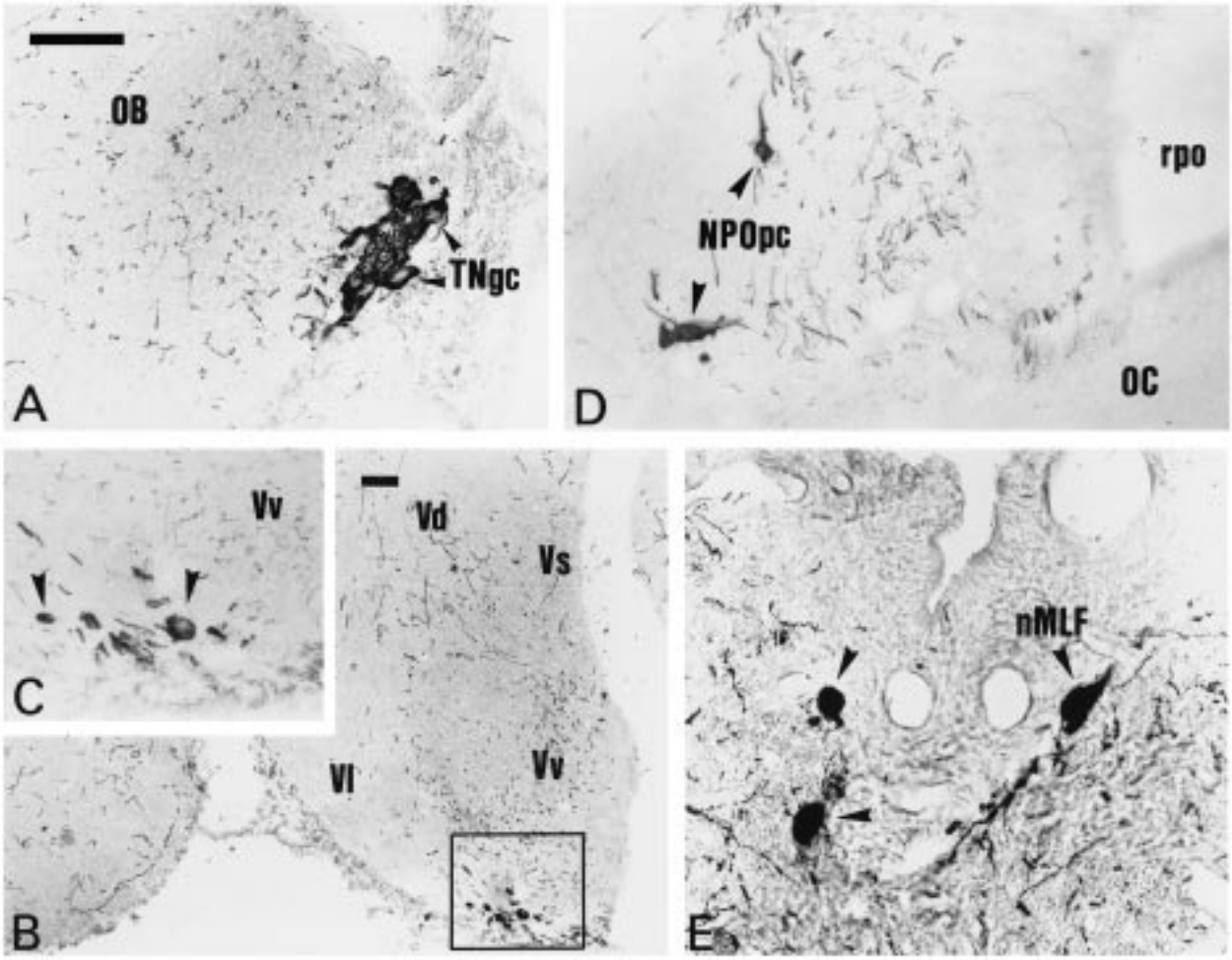

Figure 2. Photomicrographs of transverse sections of *Solea senegalensis* brain, showing GnRH-immunoreactive cells and fibres. A. Caudal olfactory bulbs. Rostralmost positive perikarya (arrowheads) in the terminal nerve (TNgc). Anti-sGnRH antiserum. B. Ventral telencephalon. Positive GnRH cells and fibres in the area ventralis. The squared area is magnified in C. Anti-sGnRH antiserum. C. Ventral telencephalon. High magnification of GnRH-immunoreactive cell bodies (arrowheads) of the pars ventralis (Vv). Anti-sGnRH antiserum. D. Preoptic area. Positive cells (arrowheads) in the pars parvocellularis of the nucleus preopticus parvocellularis (NPOpc). Anti-sGnRH antiserum. E. Synencephalon. Caudalmost positive perikarya (arrowheads) in the nucleus of the medial longitudinal fasciculus (nMLF). Anti cGnRH-II-antiserum. For other abbreviations, see Table 1. Bar scale in A represents 100 μm and is the same for C, D and E. Bar scale in B also represents 100 μm.

respectively. As in our study, in the dwarf gourami, terminal nerve cells showed strong sGnRH and a weaker cGnRH-II immunoreactivity, while midbrain tegmentum cells exhibited strong cGnRH-II and a weaker sGnRH immunoreactivity (Yamamoto *et al*. 1995). Although colocalization of different GnRH forms in the same cell masses cannot be completely excluded, this observation might be the consequence of the cross-reactivity of the antisera with more than one GnRH form in a non-competitive system such as immunohistochemistry.

Recently, it has been demonstrated in perciforms that three forms of GnRH, i.e. sGnRH, sbGnRH and cGnRH-II, are expressed at different nuclei in the brain and have separate embryonic origins (Powell *et al*. 1994, White *et al*. 1995, Gothilf *et al*. 1996, Okuzawa *et al*. 1997, Parhar 1997). Chromatographic and immunological studies suggested that the new form of GnRH (sbGnRH) could be present in other orders, such as pleuronectiforms (Idler & Everard 1987, Okuzawa *et al*. 1993), characiforms (Somoza *et al*. 1994) or gasterosteiforms (Andersson *et al*. 1995). Based on their biological activity, brain localization and presence in the pituitary, different regulatory mechanisms and functions have been postulated for each GnRH form. Terminal nerve sGnRH might function as a neurotransmitter or neuromodulator; preoptic sbGnRH seems to represent the hypophysiotrophic hormone, which stimulates the pituitary gonadotropins secretion and is involved in sex differentiation and reproduction; and midbrain cGnRH-II might be implicated in the control of reproductive behaviour (Oka 1992, Yamamoto *et al*. 1995, Gothilf *et al*. 1996, Okuzawa *et al*. 1997, Parhar 1997, Parhar & Sakuma 1997).

In Senegalese sole, abundant sGnRH- and cGnRH-II-immunoreactive fibres were detected in the preoptic-hypophysial tract, in the neurohypohysis and in the proximal pars distalis of the adenohypophysis. Immunocytochemical analysis of the adenohypophysis reveals the presence of gonadotrophs, somatotrophs and tyrotrophs in the proximal pars distalis of the Senegalese sole (Rendón *et al*.

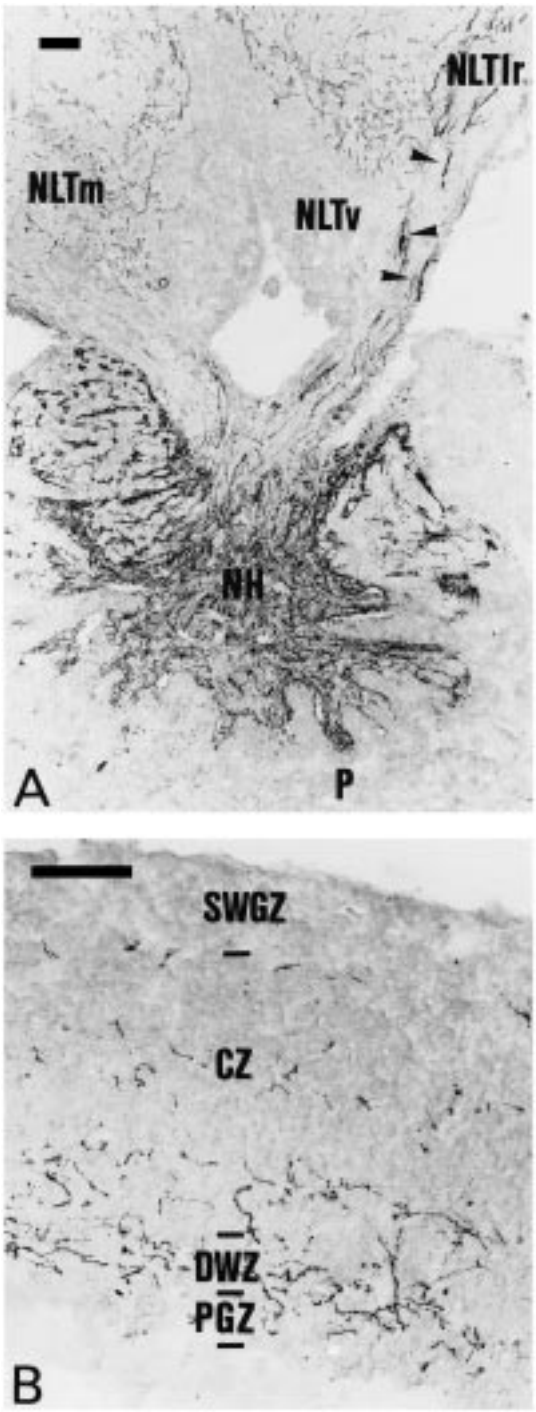

Figure 3. Photomicrographs of transverse sections of *Solea senegalensis* brain, showing GnRH-immunoreactive fibres. A. GnRH-immunoreactive fibres (arrowheads) entering the hypophysis from the ventral hypothalamus. Anti-sGnRH antiserum. B. GnRH-positive fibres in the optic tectum. Note the intense GnRH innervation of the deep white zone and the interne layer of the central zone. Anti-sGnRH antiserum. For other abbreviations, see Table 1. Bar scale in A and B represents 100 μm.

1997). The presence of both sGnRH- and cGnRH-II-immunopositive fibres have also been reported in the pituitary of other teleosts (Yu *et al*. 1988, Chang & Jobin 1994, Montero *et al*. 1994, Parhar & Iwata 1994, Kim *et al*. 1995, Parhar 1997). However, the possible role of cGnRH-II in the control of gonadotropin secretion remains speculative, and at least in seabream and masu salmon, cGnRH-II content in the pituitary of mature fish is not detectable (Powell *et al*. 1994, Kobayashi *et al*. 1997). GnRH-immunoreactive fibres in the pituitary could also regulate prolactin, growth hormone and somatolactin cells, as it has been proposed in other teleosts (Marchant *et al*. 1989, Parhar & Iwata 1994, Weber *et al*. 1997)

GnRH-immunopositive fibres, especially cGnRH-II-reactive axons, also appear distributed throughout the midbrain and hindbrain of *Solea senegalensis*. The presence of GnRH-immunoreactive fibres has also been described in the posterior brain of many teleosts (Münz *et al*. 1981, Kah *et al*. 1986, Subheadar & Rama Krishna 1988, Batten *et al*. 1990, Oka & Ichikawa 1990, Amano *et al*. 1991, Grober & Bass 1991, Yamamoto *et al*. 1995), and might originate mainly from the synencephalic GnRH cells, as it was observed in other vertebrates (Bennis *et al*. 1989, Rastogi *et al*. 1990, Wright & Demski 1991).

In conclusion, we have reported here the distribution of GnRH-immunoreactive structures in the brain of the Senegalese sole, *Solea senegalensis*, using two antisera against the sGnRH and cGnRH-II forms. This study provides a basis for future morpho-functional research on the neuroendocrine control of reproduction in Senegalese sole. Further studies should be directed to elucidate if two or more GnRH variants are expressed in the brain of this species and which GnRH form represents the real hypophysiotrophic hormone. The obtaining of specific molecular tools (e.g. cDNA probes of different GnRH forms) and specific antibodies which circumvent the problems of cross-reactivity (e.g. antibodies against different GnRH-associated peptides or GAPs) could contribute to clarify these questions.

Acknowledgements

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