ment[14-17]. It is of interest to observe that all these TRH-sensitive stimuli are also inhibited by blockade of dopamine receptors[6,7]; this finding suggests that the dopaminergic system might mediate GH response to these stimulating factors. Close interactions seem to exist between dopamine and TRH in the control of GH and PRL secretion. TRH stimulates, whereas dopamine inhibits PRL secretion in man; L-dopa also inhibits PRL release in response to TRH[6,7]. TRH has no significant effect on plasma GH levels in normal human subjects[23,24], while it is capable of inducing a significant increase of GH secretion in patients with acromegaly[25,26]. In contrast, L-dopa decreases GH release in acromegalic men[27]. These findings suggest that for both GH and PRL secretory systems a common mechanism of control might link with opposite effects dopamine and TRH. In the light of this hypothesis, it is not surprising that TRH does not inhibit LVP-induced GH secretion, since it is known that the LVP-induced GH stimulation is insensitive to blockade of dopaminergic receptors[5]. The effect of LVP on GH secretion appears to be specific (circulating levels of PRL remain unmodified after LVP administration) and peculiar, since it is not mediated by the TRH-dopamine regulatory system, at variance with the above-mentioned provocative stimuli. LVP could directly exert its action on the somatotrophs at the anterior pituitary level, as suggested by oberservations that LVP does not easily cross the blood-brain barrier[28-30]; however, the possible mediation of a hypothalamic neuroendocrine pathway unrelated to TRH and dopamine cannot be excluded.

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1 Greenwood, F.C., and Landon, J., Nature *210* (1966) 540.
2 Gagliardino, J.J., Bailey, J.D., and Martin, J.M., Lancet *1* (1967) 1357.
3 Eddy, R., Gilliland, P.F., Ibarra, J.D., Mc Murry, J.F., and Thompson, J.Q., Am. J. Med. *56* (1974) 179.
4 Chiodera, P., Coiro, V., Grichting, G., Geenen, V., and Legros, J.J., Neuroendocr. Lett. *6* (1984) 137.
5 Coiro, V., Chiodera, P., Volpi, R., Caiazza, A., Salati, G., Ferrari, P., Pignatti, D., and Rossi, G., Neuroendocr. Lett. *6* (1984) 219.
6 Muller, E.E., Nisticò, G., and Scapagnini, U., Neurotransmitters and anterior pituitary function. Academic Press, New York 1977.
7 Weiner, R.I., and Ganong, W.F., Physiol. Rev. *58* (1978) 905.
8 Morley, J.E., Psychoneuroendocrinology *8* (1983) 361.
9 Lechan, R.M., and Jackson, I.M.D., Endocrinology *111* (1982) 55.
10 Edwards, C.R.W., in: Clinics in Endocrinology and Metabolism, p. 223. Ed. G.M. Besser. Saunders, London 1977.
11 Oliver, C., Eskay, R.L., Ben-Jonathan, N., and Porter, J.C., Endocrinology *10* (1978) 481.
12 Sowers, J.R., Hershman, J.M., Skowsky, W.R., and Carlson, H.E., Hormone Res. *7* (1976) 232.
13 Jawadi, M.H., Ho, L., and De Jong, D.C., Hormone Res. *19* (1984) 91.
14 Mayer, G., and Schwinn, G., Acta endocr. (suppl.) *87* (1978) 10.
15 Maeda, K., Kato, Y., Chidara, K., Ohgo, S., Iwasaki, Y., and Imura, H., J. clin. Endocr. Metab. *41* (1975) 408.
16 Maeda, K., Kato, Y., Chihara, K., Ohgo, S., Wasaki, Y., Abe, H., and Imura, H., J. clin. Endocr. Metab. *43* (1976) 453.
17 Zanoboni, A., Zecca, L., and Zanoboni-Muciaccia, W., Clin. Endocr. *18* (1983) 233.
18 Zanoboni, A., Zanoboni-Muciaccia, W., Zanussi, C., and Baraldi, R., J. endocr. Invest. *2* (1979) 347.
19 Brown, P.M., Baccus, R., Sachs, L., Sonksen, P.H., and Wheeler, M., Clin. Endocr. *10* (1979) 481.
20 Schalch, D.S., and Parker, M.M., Nature *203* (1964) 41.
21 Noel, G.L., Suh, H.K., Stone, J.B., and Frantz, A.G., J. clin. Endocr. Metab. *35* (1972) 840.
22 Del Pozo, E., Kleinstein, J., Brun del Re, R., Derrer, F., and Martin-Perez, J., Horm. Metab. Res. *12* (1980) 26.
23 Schalch, D.S., Gonzales-Barcena, D., Kastin, A.J., Schally, A.V., and Lee, L.A., J. clin. Endocr. Metab. *35* (1972) 609.
24 Anderson, M.S., Bowers, C.Y., Kastin, A.J., Schalch, D.S., Schally, A.V., Snyder, P.J., Utiger, R.D., Wilber, J.F., and Wise, A.J., New Engl. J. Med. *285* (1971) 1279.
25 Irie, M., and Tsushima, T., J. clin. Endocr. Metab. *35* (1972) 97.
26 Faglia, G., Beck-Peccoz, P., Ferrari, C., Travaglini, P., and Ambrosi, B., J. clin. Endocr. Metab. *36* (1973) 1259.
27 Liuzzi, A., Chiodini, P.G., Botalla, L., Cremascoli, G., and Silvestrini, F., J. clin. Endocr. Metab. *35* (1972) 941.
28 Ang, V.T.Y., and Jenkins, J.S., J. Endocr. *93* (1982) 319.
29 Simon-Oppermann, C., Gray, D., Szeze Panska-Sadowska, E., and Simon, E., Am. J. Physiol. *245* (1983) R541.
30 Sorensen, P.S., Vilhardt, H., Gjerris, F., and Warberg, J., Eur. J. clin. Invest. *14* (1984) 435.

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Immunocytochemical localization of GnRH (gonadotropin releasing hormone) systems in the brain of a marine teleost fish, the sole

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Summary. The GnRH system was studied in the brain of the sole by immunocytochemistry (peroxidase-antiperoxidase method) (PAP) using antibodies to synthetic salmon GnRH (s-GnRH). Two centers containing immunoreactive cell bodies were observed in the forebrain, one located at the junction between the olfactory bulbs and the telencephalon and the other in the preoptic area. Numerous immunoreactive fibers were found, especially in the telencephalon, hypothalamus, pituitary, optic tectum and retina.
Key words. GnRH; immunocytochemistry; brain; fish; reproduction.

Our laboratory is involved in studying the reproductive biology of the sole, *Solea solea* L., in particular the neuroendocrine control of gonadotropin secretion through the gonadotropin releasing hormone (GnRH). A homologous radioimmunoassay (RIA) for salmon GnRH (s-GnRH) has recently been developed[1] for determination of the annual variations of brain and pituitary GnRH content in the sole. However, for a better interpretation of the results, an immunocytochemical study of the cerebral GnRH distribution appeared to be useful, and is described in this paper.

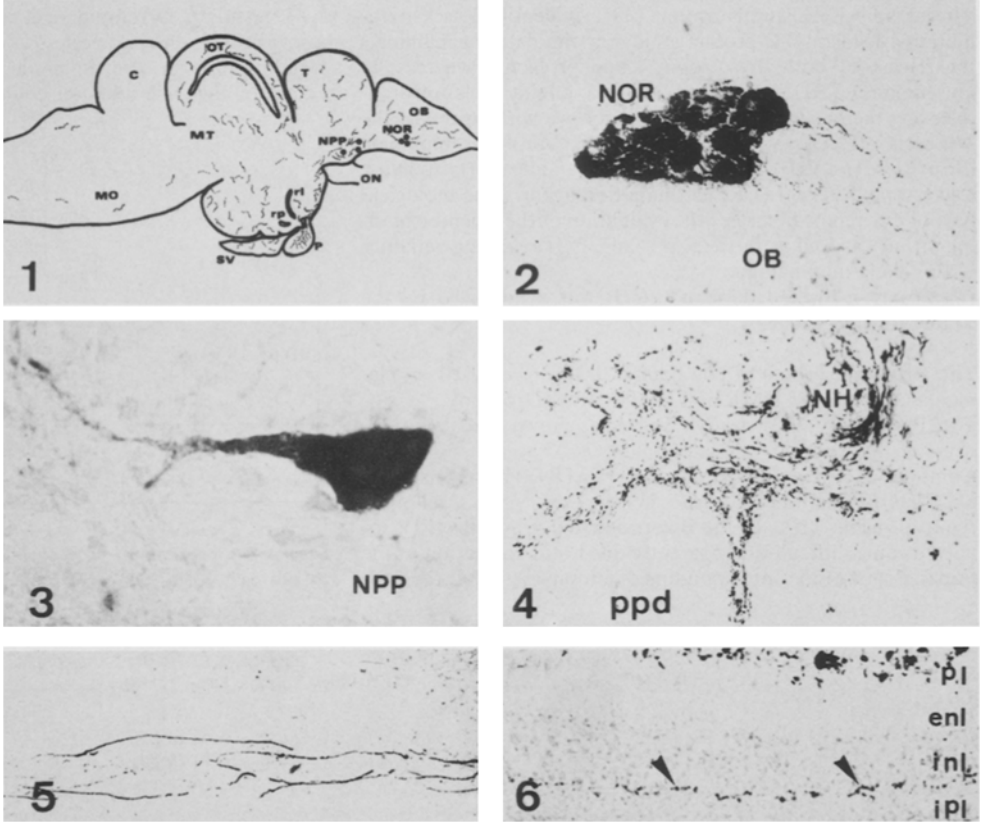
Material and methods. The soles were caught by trawling off the coast of Arcachon, with the oceanographic ship of the C.N.R.S. (Côte d'Aquitaine). On board, the fish were kept in a large tank of running seawater and transferred into aerated seawater tanks under a natural photoregime in the laboratory. Experiments were conducted on fourteen 2-year-old male and female fish. The fish were anesthetized with MS222 (SANDOZ), and the brains, pituitaries and retinae then removed and immersed in 4% formaldehyde in 0.1 M phosphate buffer at pH 7.7 for 1 h at 4°C. After rinsing for 1 h in the same buffer the tissues were

immersed in 12% sucrose phosphate buffer solution for 12 h at 4 °C and then frozen. Serial transverse and longitudinal sections were incubated for 12 h at 4 °C with the primary antibody diluted 1:1000 to 1:3000 in phosphate buffer and rinsed in the same buffer. The sections were placed for 2 h in swine IgG (DAKO, Denmark) diluted 1:200, and after rinsing the slides were incubated for 1 h in peroxidase-antiperoxidase complexes diluted 1:600 (DAKO, Denmark). The sections were then treated with a solution containing 0.025% of 3,3-diaminobenzidine tetrachloride (SIGMA) and 0.006% of hydrogen peroxide dissolved in 0.05 M Tris hydrochloride buffer at pH 7.6. The specificity of the antiserum to synthetic s-GnRH had previously been tested in a radioimmunoassay (RIA) system[1], Nunez Rodriguez (unpublished observations). Omission of one step of the reaction or replacement of the primary antiserum by rabbit normal serum confirms the specificity of the immunoreaction. Liquid phase absorption of the specific antiserum by synthetic s-GnRH led to the extinction of the reaction.

Results. Using the PAP method, immunoreactive cell bodies were observed in two regions of the forebrain. The first is located medioventrally in the caudal part of the olfactory bulbs just beside the ventral telencephalon (figs 1 and 2). This group is formed by 15–20 neurons (30–40 µm in diameter) from which numerous fibers travel rostrally in the olfactory bulbs. The caudal projection of this nucleus is not very obvious. The second group of immunopositive cell bodies is located in the ventrolateral parts of the preoptic region (figs 1 and 3). These cells emit dorsally numerous processes forming a large bundle running ventrocaudally above the optic tract (figs 1 and 5) into the ventral hypothalamus and the pituitary stalk. In the pituitary gland itself these fibers scatter in the neurohypophysis (figs 1 and 4). A large number of fibers is observed in the different areas of the telencephalon and diencephalon. A high density of immunoreactive fibers is found in the ventral hypothalamus without particular organization, except for those entering the pituitary stalk. The saccus vasculosus is also well innervated by GnRH fibers originating from the ventroposterior hypothalamus. Extensive innervation by immunoreactive GnRH fibers is found in the optic tectum. They are located mainly in the stratum album griseum but are also to be found in the other layers. More caudally, fibers are found in the ventral area of the medulla oblongata but not in the cerebellum. Numerous fibers are observed in the retina (fig. 6) close to the bipolar and amacrine cells.

Discussion. We detected two GnRH centers containing immunoreactive cell bodies in the brain of a marine teleost fish by using an antibody to synthetic salmon GnRH[2]. The specificity of this antiserum has previously been studied by RIA[1].

Radioimmunoassayable GnRH has been found in brain and pituitary extracts of the sole (Nunez Rodriguez and Breton, unpublished data) and previous studies have suggested that the primary structure of GnRH in sole is identical to that of salmon[1]. Other authors have also demonstrated the structural identity of GnRH in different teleost species[3]. In the sole, the first nucleus is located in the caudal part of the olfactory bulbs and probably corresponds to the nucleus olfactoretinalis described in the platyfish[4–6], where the relationship between this nucleus and both the olfactory system and the retina is demonstrated. The presence of GnRH in the retina of the sole suggests that a similar relationship exists in this species. By the use of LHRH antibodies[4–7] it has been demonstrated that the neurons of the second center do not form a well-defined nucleus but are scattered within the ventral preoptic area. Our studies showed that these perikarya project as far as the neurohypophysis via the ventrolateral hypothalamus. Similar observations have been reported in the platyfish[4] and the goldfish[8]. Although the presence of immunoreactive fibers in the other cerebral areas has been described in the species studied[4–8], their origin is not clear. Very little has so far been discovered concerning the functional significance of these phenomena. The nucleus olfactoretinalis has been described in the goldfish as an element of the nervus terminalis



1 Distribution of immunoreactive material in the brain and pituitary of the sole: C, cerebellum; MO, medulla oblongata; MT, midbrain tegmentum; NPP, nucleus praeopticus periventricularis; NOR, nucleus olfactoretinalis; OB, olfactory bulb; ON, optic nerve; OT, optic tectum; P, pituitary gland; rl, lateral recess; rp, posterior recess; SV, saccus vasculosus; T, telencephalon (× 6.4). *2* GnRH immunoreactive cell bodies in the caudal part of the olfactory bulb. Longitudinal section (× 200). *3* Immunoreactive neuron in the preoptic area (× 560). *4* GnRH fibers in the anterior part of the pituitary. Longitudinal section: NH, neurohypophysis; ppd, proximal pars distalis (× 120). *5* Longitudinal section of a fascicle of GnRH fibers above the optic tract (× 400). *6* GnRH fibers in the retina at the level of the bipolar and amacrine cells: enl, external nuclear layer; inl, inner nuclear layer; ipl, inner plexiform layer; pl, pigmented layer (× 320).

(terminal nerve) which mediates responses to sexual pheromones[9]. The innervation of the retina by fibers of the terminal nerve suggests that the GnRH acts as a transmitter in this retinopetal system[10]. All these data confirm the important role of this system in sexual behaviour as has been described in mammals[11]. The connection of the cell bodies described in the preoptic region with the pituitary gland suggests that this center acts more directly on the gonadotrophic function. Further studies are necessary to determine the relationship between the two centers and the multifunctional actions of GnRH.

1 Breton, B., Motin, A., Kah, O., Le Menn, F., Geoffre, S., Precigoux, G., and Chambolle, P., C.r. Acad. Sci., Paris *299* (1984) 388.
2 Sherwood, N., Eiden, L., Brownstein, M., Spiess, J., Rivier, J., and Vale, W., Proc. natn. Acad. Sci. USA *80* (1983) 2798.
3 Sherwood, N.M., Harvey, B., Brownstein, M.J., and Eiden, L.E., Gen. comp. Endocr. *55* (1984) 181.
4 Munz, H., Stumpf, W.E., and Jennes, L., Brain Res. *221* (1981) 13.
5 Munz, H., Claas, B., Stumpf, W.E., and Jennes, L., Cell Tiss. Res. *222* (1982) 323.
6 Halpern-Sebold, L.R., and Schreibman, M.P., Cell Tiss. Res. *229* (1983) 84.
7 Schreibman, M.P., Halpern, L.R., Goos, H.J.Th., and Margolis Kazan, H., J. exp. Zool. *210* (1979) 160.
8 Kah, O., Chambolle, P., Dubourg, P., and Dubois, M., Gen. comp. Endocr. *53* (1984) 115.
9 Demski, L.S., and Northcutt, R.G., Science *220* (1983) 437.
10 Stell, W.K., Walker, S.E., Chohan, K.S., and Ball, A.K., Proc. natn. Acad. Sci. USA *81* (1984) 944.
11 McCann, S.M., A. Rev. Pharmac. Toxic. *22* (1982) 515.

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TRH analogue with C-terminal thioamide group. Synthesis, receptor binding, TSH-releasing activity and α-MSH-releasing activity[1]

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Summary. A new TRH analogue containing a C-terminal thioamide group was synthesized. This peptide was shown to have receptor-binding affinity, and TSH- as well as α-MSH-releasing activities very similar to native TRH.
Key words. [Prot^3]TRH; receptor-binding affinity; TSH-releasing activity; α-MSH-releasing activity; thiopeptide.

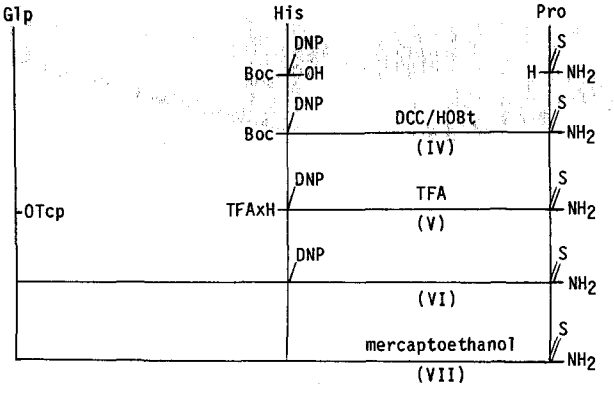
Thyrotropin-Releasing Hormone (TRH), identified as L-pyroglutamyl-L-histidyl-L-proline amide[2,3], stimulates in mammals the release of both thyrotropin[4,5] and prolactin[5] whereas, in amphibians, TRH was found to be a potent melanotropin-releasing factor[6]. In this communication we wish to report the synthesis of L-pyroglutamyl-L-histidyl-L-proline thioamide ([Prot^3]TRH) (**VII**) from proline thioamide (**III**) applying Lawesson's Reagent (LR) as thionation agent[7]. The biological part of our report describes the evaluation of the receptor-binding affinity as well as the α-MSH- and TSH-releasing activities of this TRH thioanalogue.

Chemistry. L-Proline thioamide (**III**) was synthesized by the route shown in scheme 1.

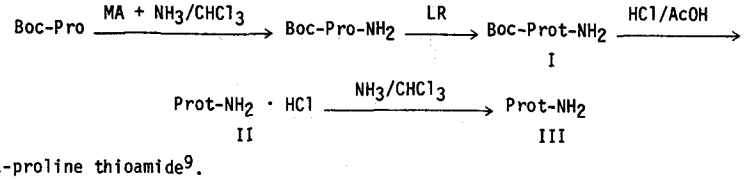
The structural proofs of compounds **I**–**III** were based on elemental analysis, ^{1}H-NMR and ^{13}C-NMR spectroscopy.

The [Prot^3]TRH (**VII**) was synthesized as shown in scheme 2.

Compound **III** was coupled with Boc-His(DNP) by means of the DCC/HOBt method[10] to give **IV** in 75% yield. Acidolytic removal, using TFA, of the Boc-protecting group from **IV** involved some difficulties, apparently due to the formation of the thioanalogue of the corresponding diketopiperazine (L-thioprolyl-L-histidyl). Diketopiperazine formation from carboxy-terminal proline dipeptide esters is well documented[11], but such a rapid cyclization of a dipeptide amide is less common[12]. The extent of this side reaction could be minimized, however, by



Scheme 2. Preparation of L-pyroglutamyl-L-histidyl-L-proline thioamide[9].



Scheme 1. Preparation of L-proline thioamide[9].