Thyrotropin-releasing hormone does not inhibit lysine vasopressin-induced growth hormone secretion in normal men

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Summary. In order to establish whether thyrotropin-releasing hormone (TRH) inhibits lysine-vasopressin (LVP)-induced growth hormone (GH) release, six normal men were tested with LVP alone or in combination with TRH. LVP strikingly increased serum GH levels; this response was not altered by TRH. These results indicate that in man TRH is not involved in the control of GH secretion in response to LVP.

Key words. Thyrotropin-releasing hormone; lysine vasopressin; growth hormone.

Treatment of normal human subjects with lysine-vasopressin (LVP) strikingly increases serum growth hormone (GH) concentrations[1-5]. Until now, the mechanism of action of LVP has not been elucidated. Previous studies carried out in order to determine whether dopamine or opioid peptides, which mediate the effect of other stimuli for GH secretion[6-8], also participate in the regulation of LVP action, failed to provide evidence for the involvement of these neuroendocrine pathways[5].

Recently, a high density of thyrotropin-releasing hormone (TRH)-positive perikarya has been demonstrated with immunohistochemical methods in the paraventricular nucleus[9], where vasopressin is synthesized[10]; furthermore, high concentrations of radioimmunoassayable TRH have been detected in the posterior pituitary[11], where vasopressin is stored[10]. In addition, in vivo studies have shown that TRH administration in euthyroid and hypothyroid human subjects modifies plasma concentrations of vasopressin[12,13]. These in vitro and in vivo findings suggest the possible existence of interactions between these two neuropeptides. Since some GH-releasing stimuli such as physical exercise, insulin-induced hypoglycemia or the administration of L-dopa, bromocriptine, clonidine and arginine are inhibited by TRH in humans[14-19], we wondered whether LVP and TRH could interact in the regulation of GH secretion in man.

The present study was undertaken in order to verify whether LVP-induced GH increase can be inhibited by the concomitant administration of TRH, and thus, whether LVP action is comparable with that of other stimuli for GH secretion, at least on the basis of its sensitivity to TRH.

Materials and methods. Six normal male volunteers, aged 24–34 years, participated in the study. All of them gave informed consent. Subjects were within 10% of their ideal b.wt, and were not affected by endocrine, metabolic or other diseases. None of them was taking any drug before and during the period of this study. Each subject was tested twice; tests were performed after an overnight fast, while the men were in a recumbent position. One test (control test) consisted of the i.v. injection of LVP. At 09.00 h on the day of the experiment, two 19-gauge i.v. indwelling needles were inserted in the antecubital veins of opposite arms and were kept patent by slow infusion of normal saline (NaCl 0.9%). One catheter was used for blood sampling, the other for drug administration. The first blood sample (time 0) was taken 20 min after the insertion of the needle and was followed by the injection of a bolus (0.06 IU/kg) of synthetic lysine-8-vasopressin over a 5-min period. Further blood specimens were withdrawn 15, 30, 45, 60 and 90 min after LVP injection. Serum samples were separated and frozen at −20 °C and were used for GH assay.

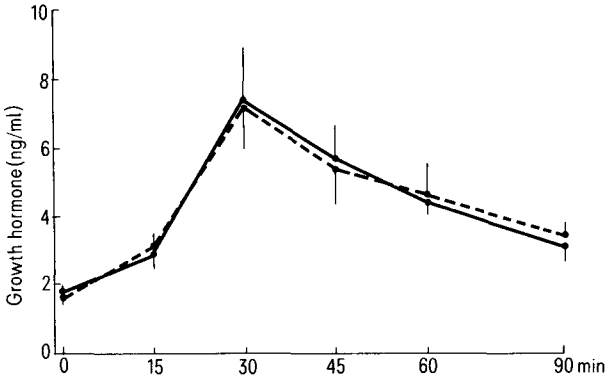
When the effect of TRH on the response of GH to LVP was studied (experimental test), a similar procedure to that used in the control test was followed, except for the administration of TRH. TRH was given as an i.v. bolus of 50 μg (10 min before the injection of LVP), plus an additional infusion for 90 min of 1 mg dissolved in 250 ml of normal saline, which was started just after LVP administration. These tests were performed in each subject at weekly intervals and in random order. Serum concentrations of GH were measured with a specific radioimmunossay[20], using the double antibody technique; samples from the same subject were evaluated in duplicate and in the same assay. The limit of sensitivity was 0.25 ng/ml.

Data were statistically analyzed using Student's t-test and Mann-Whitney's U-test, as appropriate. All results are expressed as mean ± SE.

Results. The administration of LVP strikingly increased serum concentrations of GH in all subjects, reaching maximal peak levels at 30 min after the injection (fig.). Treatment with TRH neither modified basal concentrations of GH, nor counteracted the effect of LVP on the release of GH. In both tests, side effects such as nausea or gastric cramps were observed in three out of six subjects after the injection of LVP. Subjects with (3) and without side effects (3) had similar basal concentrations of GH (1.6 ± 0.4 ng/ml vs 2.0 ± 0.2 ng/ml) and GH peak response to LVP (7.8 ± 2.1 ng/ml vs 7.1 ± 2.6 ng/ml). Blood pressure remained constant in all the men during the tests.

Discussion. The increase of serum circulating levels of GH after LVP administration cannot be considered an aspecific effect of the stress of the experiment, since both basal levels of GH and peak concentrations of GH in response to LVP were similar in all subjects, regardless of the presence of side effects. Furthermore, serum levels of prolactin, which are also sensitive to stress[21], did not change in our subjects after LVP injection (unpublished data), as also observed in previous studies[22].

The results of this study clearly show that the i.v. administration of TRH does not affect GH response to LVP. TRH failure cannot be attributed to the use of an inadequate dose or to the route of administration, since similar protocols were used successfully by others in order to inhibit GH release in response to various stimuli[15-18]. Our data speak against a role of TRH in the regulation of LVP action. In this respect, the GH stimulating effect of LVP differs from that of other stimuli, such as physical exercise, or the administration of L-dopa or arginine, or insulin-induced hypoglycemia, which are inhibited by TRH treat-



Effect of LVP (0.06 IU/kg) (●——●) or LVP plus TRH (50 μg as a bolus i.v. 10 min before LVP administration plus 1 mg/90 min infusion) (●– – –●) on serum concentrations of GH in six normal male subjects. Each point represents the mean ± SE of the observations.

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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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