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Radioimmunoassay of growth hormone-releasing hormone (GHRH) with a polyclonal antibody against synthetic GHRH(1-29)-Gly_4-Cys-NH_2: method and clinical studies

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Summary

A radioimmunoassay (RIA) for growth hormone-releasing hormone (GHRH) using a polyclonal antibody against synthetic GHRH(1-29)-Gly_4-Cys-NH_2 has been developed. The antiserum (RBM105) showed full cross-reactivity with GHRH-(1-44)NH_2, GHRH-(1-40)OH, GHRH-(1-37)OH and GHRH-(3-44)NH_2, and probably recognized the region of Ala^4 to Lys^{12} of GHRH. Since the sensitivity of the GHRH RIA was 1.5 pg/tube, the lowest detectable plasma level was 5 ng/l when an extract of 0.3 ml of plasma per tube was used. On gelfiltration chromatography, the GHRH immunoreactivity of normal plasma was eluted in the same position as synthetic GHRH. The plasma GHRH concentration in healthy subjects was 20.5 ± 6.5 ng/l (mean ± SD), and in patients with hypothalamic disorders was 17.4 ± 2.0 ng/l. In contrast, the plasma GHRH level in hemodialysis-dependent, chronic renal failure (CRF-HD) patients (38.7 ± 13.1 ng/l) was significantly higher than normal. The acromegalic patients were 24.3 ± 11.9 ng/l, except for one patient with ectopic GHRH syndrome (990 ng/l): his plasma GHRH level reached 7,100 ng/l during operation, and then decreased logarithmically to 70 ng/l after 6 h. Somatostatin at concentrations of 10 and 1,000 nmol/l significantly suppressed

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(GHRH release) from primary culture cells of the GHRH-producing tumor from 17.3 ± 0.92 ng/2×10^5 cells to 9.98 ± 3.61 and 4.32 ± 1.01 ng/2×10^5 cells, respectively after 48 h.

These data indicate that this GHRH RIA is useful for determining the plasma GHRH concentration in normal and diseased states and also for in vitro studies of GHRH release.

Introduction

Since the discovery of growth hormone-releasing hormone (GHRH) [1,2], there have been several reports of preparation of antisera aganist synthetic GHRH to establish a radioimmunoassay (RIA) and immunohistochemical methods for GHRH localization and measurement. However, the results obtained by RIA and in immunohistochemical studies have not been consistent, due to differences in the immunological properties of the anti-GHRH sera [3]. Therefore, it is important to characterize the antisera by determining their specific sites of recognition of the GHRH molecule. Moreover, before RIA determination of plasma GHRH, non-specific substances that interfere with the assay should be removed. In this paper, we describe an RIA for GHRH with a polyclonal antibody against synthetic GHRH(1-29)-Gly_4-Cys-NH_2 recognizing the N-terminal part of the GHRH molecule. We also report plasma GHRH levels in normal subjects and patients with various diseases and the in vitro effect of somatostatin on GHRH release from cultured tumor cells producing GHRH.

Materials and methods

Subjects

Plasma GHRH levels were determined in 34 healthy subjects of 20–40 yr old with no obesity or endocrine disorders, 8 patients with hypothalamic disorders (5 suprasellar germinomas, 2 craniopharygiomas and 1 meningioma), 15 patients with hemodialysis-dependent, chronic renal failure (CRF-HD), and 15 acromegalics. The changes in plasma GHRH and GH concentrations in a patient with ectopic GHRH syndrome [4–6], before, during and after resection of his pancreatic tumor were also measured. Blood samples were drawn from ante-cubital vein into polypropylene tubes containing aprotinin (500 KIU/ml) with EDTA (1.2 mg/ml), promptly placed in an ice bath, and centrifuged at 3,000 rpm for 20 min at 4 °C. The plasma was then stored at −40 °C until assay. For characterization of GHRH immunoreactivity in plasma, samples of 40 ml of plasma were obtained from a healthy subject.

Preparation of anti-GHRH serum

Polyclonal antibody was raised in rabbits by serial subcutaneous injections of GHRH conjugates. The GHRH conjugates were prepared by coupling synthetic