CLINICAL APPLICATION OF GASTROENTEROPANCREATIC HORMONES (GLUCAGON AND GASTRIN) RADIOIMMUNOASSAY DEVELOPED AT IPEN - CNEN / SP: AN OVERVIEW

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Summary

A pancreatic glucagon and gastrin radioimmunoassays employing specific antiseruns were developed at IPEN. Both of the hormones were radioiodinated by the chloramine T technique and purified on QAE-Sephadex A25 to a specific activity of around 250 uCi/ug.

The standard curves allowed measurements from 2 to 150 pmol/l with acceptable intra and inter-assay precision.

Fasting glucagon levels determined in diabetics, obese subjects, acromegalics and patients with Cushing's syndrome were greater than in normals (6.3 ± 26 pmol/l; mean ± SD). Fasting gastrin values estimated in chagasics, patients with pernicious anemia, chronic renal failure and an unique patient with Zollinger-Ellinson's syndrome were greater than in normals (16.4 ± 15 pmol/l),, while in the patients submitted to gastrectomy the gastrin leves were very low, as expected.

INTRODUCTION

Although glucagon had been the second hormone measured by the radioimmunological technique of Berson and Yalow in 1959 (1), only after a decade it became possible to measure this hormone in the human circulation with valid results (2).

The reasons by which the development of glucagon assay was delayed were its degradation, weak antigenicity and cross-reaction between the pancreatic and the gut hormone. These technical obstacles were eliminated by the use of an antienzyme to prevent any hormonal degradation and by the production of highly sensitive and specific glucagon antiseruns (3-5).

The development of a radioimmunoassay for pancreatic glucagon at IPEN in the begining of 1980's (6) was made possible by the support that authorities in this field provided in training and supplying of biological reagents. Thus, the contribution of Drs. SR Bloom from the Royal Postgraduate Medical School, London, LG Heding from Novo Research Institute, Copenhagen and RH Albuquerque from Sarah Kubitscheck Hospital, Brasilia,deserve particular citation.

The standardization of glucagon radioimmunoassay at our laboratory was followed by other specific assay developed for gastrin (7). This radioimmunoassay was initially established in 1968 (8,9) and set up by other workers in 1970's (10-12).

The present report describes the highly sensitive and specific glucagon and gastrin radioimmunoassays developed in the last years at IPEN, employing carefully prepared tracers (13-15) with great stability after QAE-Sephadex purification and specific antiseruns. In addition, the validity of the assays was confirmed by its clinical application measuring fasting levels of those hormones in patients with different disorders in comparison with normal subjects.

These radioimmunoassays were developed in cooperation with the Endocrinology and Gastroenterology Departments of the Medical School of São Paulo University (FMUSP).

MATERIAL AND METHODS

The radioimmunoassays were developed according to principles described by Dr. SR Bloom's group (5,12), employing twice crystallized porcine glucagon (Novo Research Institute) and synthetic human gastrin, hG17 (Research Plus Laboratories) for iodination. The standard hormones were supplied by the Medical Research Council (porcine glucagon 69/194 and synthetic hG17 68/439). The specific antiseruns (C terminal reagents) raised in rabbits were supplied by Drs. SR Bloom for glucagon (RC55) and JH Walsh for gastrin (1611).

These hormones were radioiodinated by a modification of the chloramine T technique and the monoiodinated hormonal components were purified by anion exchange chromatography in QAE - Sephadex A 25, (6,14).

The assays were routinely performed in a total volume of 0.8 ml, using a veronal buffer, pH 8.0. Increasing concentrations of standard hormones (from zero to 100 nmol) diluted in hormone free plasma in a volume equal to the unknown samples (0.2 ml) were used in the standard curve. The free plasmas were prepared from pooled, time-expired blood bank plasma treated by repeated freezing and thawing and then incubated at 56ºC for 30 min to lower the glucagon content or extrated with 5% charcoal (Merck Reinst) to eliminate the endogenous gastrin. A volume of 0.1 ml anti-seruns with appropriate dilution and 0.1 ml of the [^{125}I] hormones were added to the reaction mixture and incubated for 4 to 5 days at 4ºC. The antibody bound and free [^{125}I] hormones were separated by adsorption of the latter to the charcoal. The percentage of [^{125}I] hormones bound to its antiseruns was calculated in relation to the total radioactivity.

The specific activity of the tracers used in the assays was determinated by the method of self-displacement (16).

The precision of the radioimmunoassays was analyzed estimating the within and between-assay reproductibility of samples containing high, medium and low hormonal levels.

Fasting levels of plasma glucagon were measured in 58 individuals divided into five groups consisting of 19 normals, 20 diabetics, 10 obeses, 7 active acromegalics and two patients with Cushing's syndrome. Fasting gastrin values were estimated in 124 individuals divided into six groups of 64 normals, 27 chagasics, 5 patients with pernicious anemia,13 with chronic renal failure, 14 submitted to gastrectomy and one patient with Zollinger-Ellison's syndrome.

Statistical analysis were made by Student's t-test for glucagon unpaired observations and by the Mann-Whitney U test for gastrin data.

RESULTS

Fractions corresponding to the hatched area from the peaks of the anion-exchange chromatograms in the [^{125}I] hormones purification which presented higher binding after a brief incubation with concentrated antiserum, were pooled and stored at -20ºC until use as tracers in routine radioimmunoassays (Fig.1).

Labeled hormones were stable for more than three months and had similar specific activities ranging from 200 to 300 uCi/ug.

The displacement of the tracers by the hormonal standards prepared in the hormone-free plasmas is showed in figure 2. The lower limit of detection in each assay was 2.0 and 3.5 pmol of gastrin and glucagon per ml, respectively. Usual radioimmunoassay standard curves allowed the measurement from 2.0 to 125.0 pmol/l of gastrin and from 3.5 to 150.0 pmol/l of glucagon.

Within-assay precision studies gave coefficients of variation (CV) of 14.9, 5.1 and 6.3 % for glucagon samples of low, medium and high concentrations (n=18), respectively. The correspondents CVs for within-assay gastrin samples were 6.1, 3.6 and 11.9 % (n=25). In the between-assay analysis, the CVs determined for

the similar levels were 10.7, 4.6 and 5.6 % for glucagon plasmas (n=5) and 6.9, 3.2 and 10.3 % for gastrin samples (n=4).

Figures 3 and 4 illustrates respectively the ranges of glucagon and gastrin values obtained on overnight-fasting samples from the studied groups. Basal mean glucagon and gastrin concentrations were significantly higher in all groups of patients compared to normals ($p < 0.001$ and 0.05, respectively), except in the gastrectomized group in which the gastrin levels were significantly lower ($p < 0.05$), as expected. In the cases of Cushing's and Zollinger-Ellison's no statistical analysis was done.

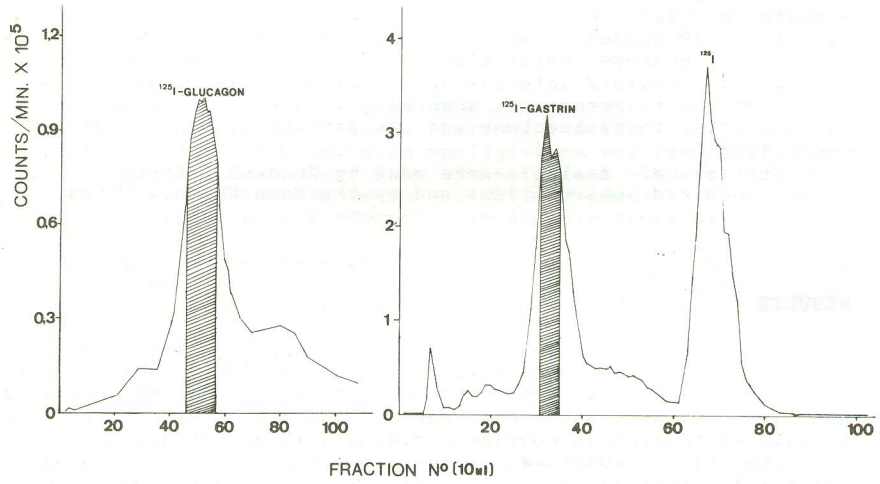

Fig 1. Purification of iodinated hormones by QAE - Sephadex A25.

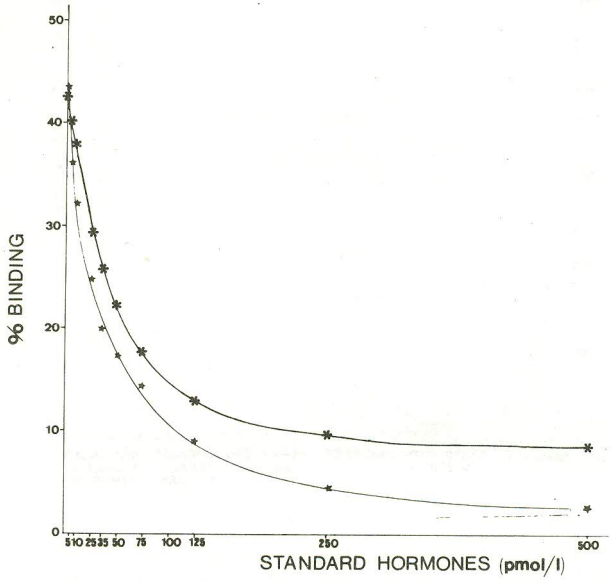

Fig. 2. Typical examples of standard curves obtained in the glucagon (*——*) and gastrin (★——★) radioimmunoassays.

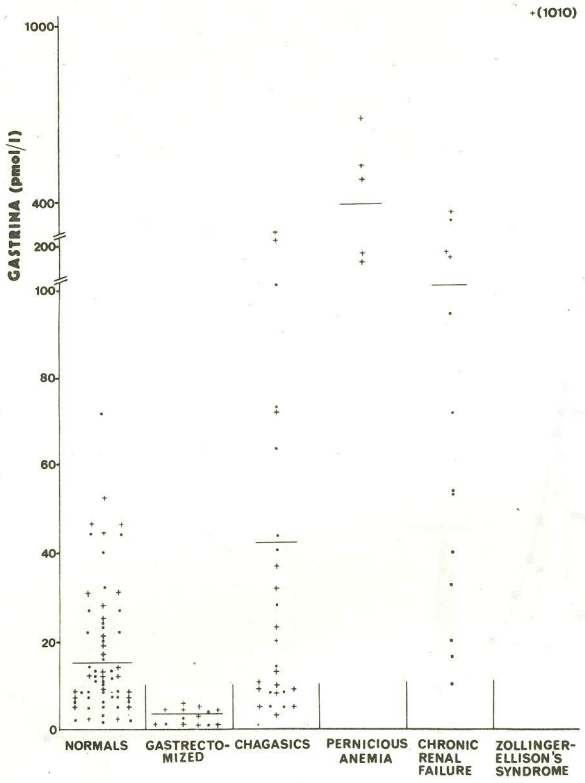

Fig.3. Fasting plasma pancreatic glucagon concentrations (mean ± SD) in normal subjects (6.3 ± 2.6 pmol/l), diabetics (15.8 ± 5.2 pmol/l), obeses (15.8 ± 3.1 pmol/l), acromegalics (14.3 ± 3.2 pmol/l) and patients with Cushing's syndrome. The mean for each group is indicated.

Females are indicated by squares (▪) and males by crosses (+).

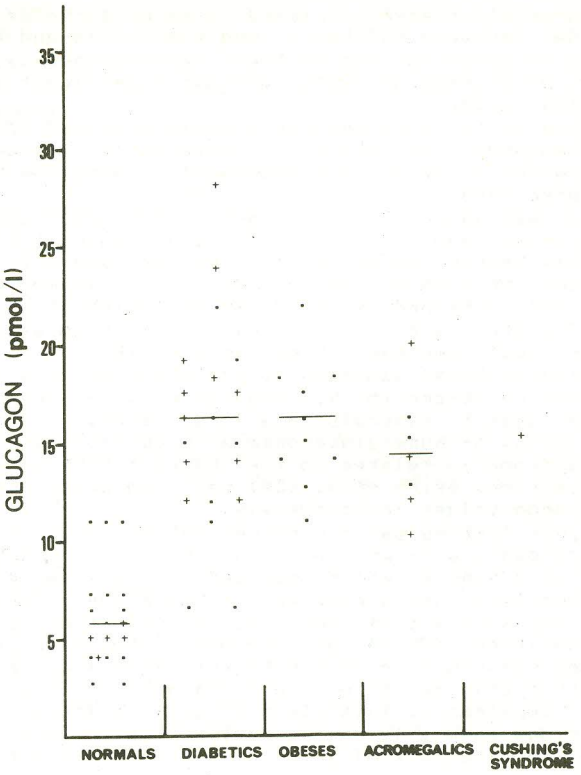

Fig. 4. Fasting circulant gastrin concentrations (mean ± SD) in normal subjects (16.4 ± 15.0), gastrectomized (3.0 ±2.3), chagasics (43.1 ± 63.7), patients with pernicious anemia (390.4 ± 219.1), chronic renal failure (107.2 ± 109.0) and Zollinger-Ellison's syndrome. The mean for each group is indicated.

Females are indicated by squares (▪) and males by crosses (+).

DISCUSSION

The hormonal tracers purified by anion exchange chromatography in QAE Sephadex A25 has a long shelf-life and high specific activity suitable for use in their radioimmunoassays even after 3 months of storage at -20ºC, without significant alterations in the binding curves.

Regarding to the hormone-free plasmas prepared to dilute the hormonal standards, they are easily obtainable, producing standard curves which allowed the measurement of very low levels with acceptable precision.

Fasting glucagon levels determined in normal subjects were similar to those reported in the literature using the same RCS5 antiserum (5). Fasting normal gastrin values ranged in accordance with previous radioimmunoassay report (17) performed with other antibody (1296) obtained by Dr. JH Walsh, which also recognizes all forms of gastrin and to bind similarly to them as compared to the antibody (1611) employed in our assays (18).

The greater basal glucagon levels observed in diabetics, as also reported by others (5,19), suggest reduced suppressibility of the alpha-cells to hyperglycemia in diabetes.

Our finding of hyperglucagonemia in obesity, acromegaly and Cushing's syndrome is related to the insensitivity of alpha-cells to insulin action. Seino et al (20) has also described elevated fasting glucagon values in acromegaly.

The higher fasting gastrin concentrations found in chagasics are in accordance with previous studies (21,22) and are due to the gastric acid hyposecretion observed in these pacients (21).

The hypergastrinemia observed in the chronic renal failure and pernicious anemia confirmes previous report of raised levels in these conditions (23,24) and are associated with achlohydria, although some reports have failed to establish a clear connection between gastric acid secretion and serum gastrin in patients with this renal impairment. This elevated gastrin found in patients with pernicious anemia is due in part to hyperplasia of G-cells and partly to the lack of acid inhibition of gastrin release (11).

Finally, the very high level of gastrin observed in the Zollinger-Ellison's syndrome, caused by a gastrin-secreting pancreatic tumor, and the very low levels observed in the gastrectomized subjects validate the assay, confirming its adequacy in the measurement of a broad hormonal range in clinical conditions.

CONCLUSION

The glucagon and gastrin radioimmunoassays developed at IPEN in cooperation with clinical departments of the FMUSP became possible the report of studies about the understanding of the physiology and pathophysiology of these hormones (6,25-27).

Furthermore, other clinical and experimental applications of this radioimmunological technique in colaboration with other Medical Schools are in progress.

ACKNOWLEDGMENTS

We wish to express our thanks to the Drs. FP Cesar, JMR Zeitune, LEA Troncon, L Romanello and WM Machado for the supply of unknown specimens, to Dr. OZ Higa and to Miss LH Lin for their valuable help.

This study was supported in part by a grant provided by Novo Research Institute, Copenhagen, a grant from FAPESP, São Paulo and a grant from CNPq, São Paulo (nº 405-557/87).

REFERENCES

1 . Unger RH, Eisentraut AM, McCall MS, Keller S, Lanz HC, Madison LL. Glucagon antibodies and their use for immunoassay of glucagon. Proc. Soc. Exp. Biol. Med. 1959; 102: 621-3.
2 . Aguilar-Parada E, Eisentraut AM, Unger RH. Pancreatic glucagon secretion in normal and diabetic subjects. Amer. J. Med. Sci. 1969; 257: 415-9.
3 . Unger RH, Aguilar-Parada E, Muller WA, Eisentraut AM. Studies of pancreatic alpha cell function in normal and diabetic subjects. J. Clin. Invest. 1970; 49: 837-48.
4 . Heding LG. Radioimmunological determination of pancreatic and gut glucagon in plasma . Diabetologia 1971; 7: 10-9.
5 . Alford FP, Bloom SR, Nabarro DN. Glucagon lavels in normal and diabetic subjects: Use of a specific immunoabsorbent for glucagon radioimmunoassay. Diabetologia 1977; 13: 1-6.
6 . Borghi VC, Wajchenberg BL, Albuquerque RH. Evaluation of a sensitive and specific radioimmunoassay for pancreatic glucagon in human plasma and its clinical application. Clin. Chim. Acta 1984; 136: 39-48.
7 . Peig MG, Bettarello A, Borghi VC, Desenvolvimento da técnica de radioimunoensaio para dosagem de gastrina no soro humano In: Abstracts of the I Reunião Anual da Federação das Socie - dades de Biologia Experimental, São Paulo, 1986: 221.
8 . Odell WD, Charters AC, Davidson WD, Thompson JC. Radioimmu - noassay for human gastrin using unconjugated gastrin as an antigen. J. Clin. Endocr. 1968; 28: 1840-2.
9 . McGuigan JE. Immunochemical studies with synthetic human gastrin. Gastroenterology 1968; 54: 1005-11.
10. Yalow RS, Berson SA, Radioimmunassay of gastrin. Gastroente - rology 1970; 58: 1-14.
11. Walsh JH. Radioimmunoassay of gastrin. In: Nuclear Medicine "in vitro". B Rothfeld, ed. Lippincott, Philadelphia ,1974 : 231-48.
12. Russell RCG, Fielding LP, Bloom SR, Bryant MG. Current pro - blems in the measurement of gastrin release. A reproducible

measure of physiological gastrin release. Postgrad. Med. J. 1976, 52: 645-50.
13. Borghi VC, Wajchenberg BL, Evaluation of glucagon stability on storage for radioiodination purposes. Horm. Metab. Res. 1982; 14:384-5.
14. Ginabreda MGP, Borghi VC, Bettarello A. Preparation of radioiodinated gastrin for radioimmunoassay. Arq. Biol. Tecnol. 1987; 30:41, abstract.
15. Ginabreda MGP, Borghi VC, Bettarello A. Quality control of radioiodinated gastrin for radioimmunoassay. Arq. Biol. Tecnol. 1988; 31: 163, abstract.
16. Morris BJ. Specific radioactivity of radioimmunoassay tracer determined by self-displacement: A re-evaluation. Clin. Chim. Acta 1976; 73: 213-6.
17. Walsh JH. Clinical significance of gastrin radioimmunoassay. Semin. Nucl. Med. 1975; 5: 247-54.
18. Jaffe BM, Walsh JH. Gastrin and related peptides. In: Methods of hormone radioimmunoassay. BM Jaffe, HR Berman, eds. Academic Press, New York, 1979: 455-77.
19. Heding LG, Rasmussen SM. Determination of pancreatic and gut glucagon like immunoreactivity (GLI) in normal and diabetic subjects. Diabetologia 1972; 8: 408-11.
20. Seino Y, Ikeda M, Kurahachi H, Taminato T, Sakurai H, Goto Y, Inoue Y, Kadowaki S, Mori K, Imura H. Failure to supress plasma glucagon concentrations by orally administered glucose in diabetic patients after treatment. Diabetes 1978; 27: 1145-50.
21. Troncon LEA, Oliveira RB, Meneghelli UG, Dantas RO, Godoy RA. Fasting and food-stimulated plasma gastrin levels in chronic Chaga's disease. Digestion 1984; 29: 171-6.
22. Mott CB, Avaliação funcional do pâncreas exócrino e da liberação de hormônios gastro-êntero-pancreáticos em portadores de doença de Chagas crônica, tese, Faculdade de Medicina da Universidade de São Paulo, 1985.
23. Korman MG, Laver MC, Hansky J. Hypergastrinemia in chronic renal failure. Br. Med. J. 1972; 1: 209-10.
24. Ganguli PC, Cullen DR, Irvine WJ. Radioimmunoassay of plasma gastrin in pernicious anemia, achlorhydria without pernicious anemia, hypochlorhydria, and controls. Lancet 1971; 1: 155-8.
25. Wajchenberg BL, Cesar FP, Leme CE, Borghi VC, Souza ITT, Neto DG, Coy DH, Schally AMC, Schally AV. Dissociated effects of somatostatin analogs on arginine-induced insulin, glucagon and growth hormone release in acromegalic patients. Horm. Metab. Res. 1983; 15:471-4.
26. Borghi VC, Wajchenberg BL, Cesar FP. Plasma glucagon suppressibility after oral glucose tolerance. Metabolism 1984; 33: 1068-74.
27. Zeitune JMR. Resposta do esfíncter inferior do esôfago e da gastrinemia à acidificação do antro gástrico em indivíduos normais e em pacientes com megaesôfago chagásico, tese. Faculdade de Medicina da Universidade de São Paulo, 1987.