EVIDENCE FOR BINDING OF GASTRIN TO LIGANDIN, A CELL CYTOSOL PROTEIN

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1. Introduction

Recent studies have indicated that gastrin is rapidly removed from the bloodstream [1,2] and is concentrated in the intestines [3,4] and to a lesser degree in kidney and liver [5]. It is not clear whether this reflects interaction with a target receptor or binding to a degradative enzyme or transport protein. We have examined binding of gastrin to ligandin, a basic protein isolated from supernates of human and rat liver, kidney and intestinal homogenates which is known to bind organic anions, cortisol metabolites, and certain carcinogens [6–8].

2. Materials and methods

Synthetic human gastrin was labelled with ^{125}I (Radio Chemical Centre Ltd., Amersham, England) to a spec. act. of ± 1500 mCi/mg [9], and purified prior to use by Sephadex chromatography. Aliquots (2×10^6 cpm) ^{125}I labelled-gastrin (± 0.7 ng) were added to 5 ml of 100 000 *g* supernate of a 25% homogenate of rat liver prepared in 0.25 M sucrose/0.01 M phosphate buffer pH 7.4 and the mixture was chromatographed on a 100 × 2.5 cm G100 Sephadex column. Elution was carried out using the same buffer and a pump-driven upward flow system at a flow rate of 15 ml/hr. The protein concentration of eluted fractions was estimated at 280 nm using a Unicam spectrophotometer model SP 1700. Radioactivity of eluted fractions was estimated on a Packard Autogamma spectrometer. The column was characterised by addition of blue dextran and ^{125}I-labelled albumin [10]. Labelled gastrin was also passed through the column in the absence of liver supernate. The ligandin peak was identified by its binding with sodium sulfobromophthalein (BSP) and also by immuno-diffusion against rabbit anti-rat liver ligandin prepared as described by Fleischner et al. [7]. The displacement of radioactive gastrin from ligandin was measured by addition of a gross excess (100 μg) of non-radioactive synthetic human gastrin to the supernate prior to chromatography. Binding of ^{125}I-labelled glucagon and insulin to ligandin was also examined. In other experiments unlabelled synthetic human gastrin was added to liver supernate and the gastrin content of the chromatographed samples estimated by radioimmunoassay [11], using an antiserum with a high affinity (3.6×10^{10} L/mol) [12] for synthetic human gastrin.

3. Results and discussion

The elution profile of ^{125}I-labelled albumin and BSP are shown in fig.1 and correspond to those previously described [6,13]. Labelled gastrin eluted at approx. 3 times the void vol of the column. Only the major BSP binding peak contained ligandin as measured by immunodiffusion analysis. Fig.2 shows the elution profile obtained when ^{125}I-gastrin was added to the supernate of 25% liver homogenate. Approximately 80% of the radioactively labelled gastrin was eluted in the peak containing ligandin. Furthermore, this peak is clearly distinguished from that of gastrin itself and gastrin bound to other proteins, for example albumin. Addition of an excess of cold gastrin to the liver supernatant, decreased recovery of radioactive gastrin in the ligandin peak to less than 40%. It is thus apparent that labelled gastrin bound to ligandin could be

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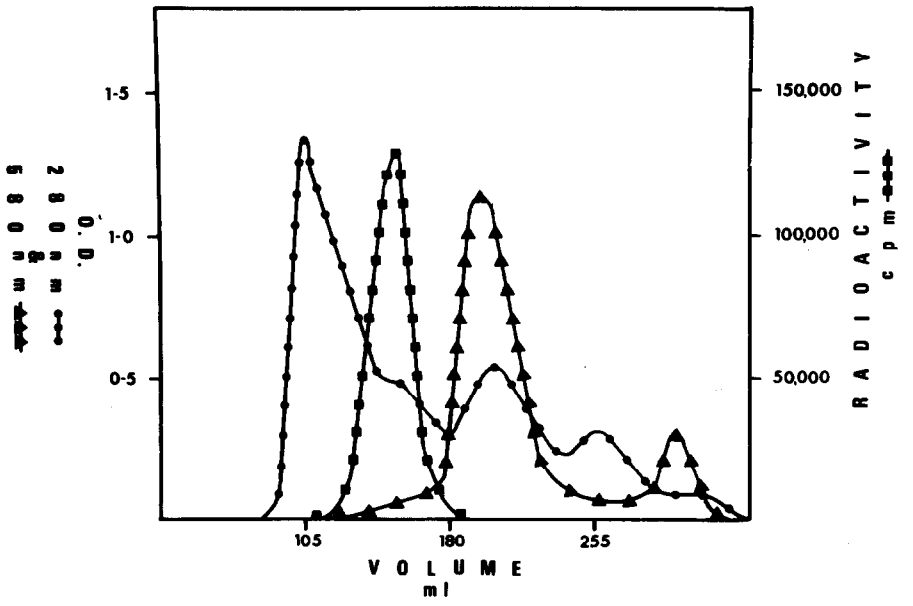

Fig.1. Sephadex (G100) elution profile of 100 000 *g* supernate of 25% homogenate of rat liver containing ^{125}I-labelled albumin (■——■) and BSP (▲——▲) (See text for details of chromatography).

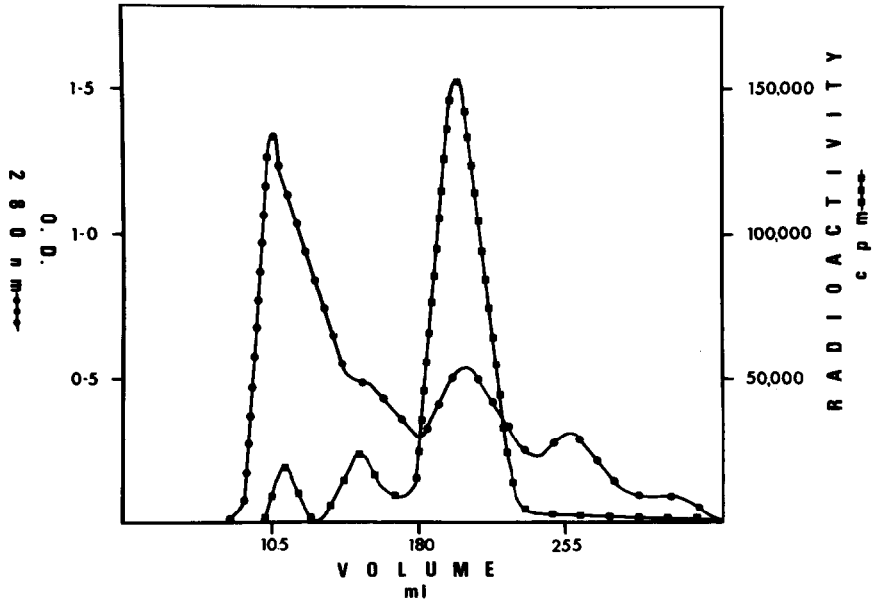

Fig.2. Sephadex (G100) elution profile of 100 000 *g* supernate of 25% homogenate of rat liver with added ^{125}I-labelled gastrin (■——■).

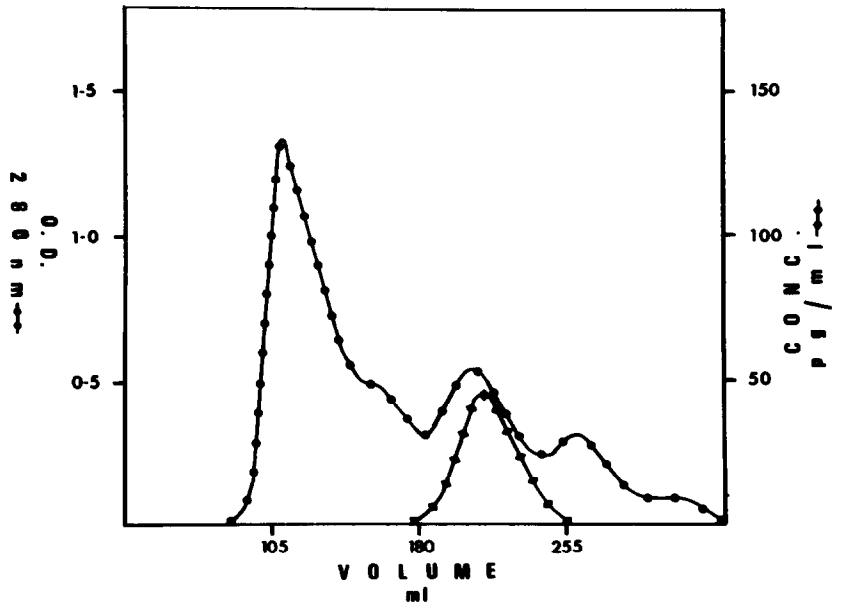

Fig.3. Sephadex (G100) elution profile of 100 000 *g* supernate of 25% homogenate of rat liver with added unlabelled gastrin (♦——♦). Gastrin in eluates was measured by radioimmunoassay.

displaced by addition of cold gastrin to the liver supernate prior to chromatography. The fact that 40% of the label was still recoverable in the ligandin peak may reflect alteration of the labelled gastrin molecule either during the process of labelling, or its coupling to ligandin, so that cold gastrin competes unfavourably with binding sites to cause displacement. Radioimmunoassay of the eluted material following addition of unlabelled gastrin to liver supernate also appears to demonstrate binding of gastrin to ligandin as shown in fig.3. The finding that gastrin bound to ligandin was measurable by immunoassay may reflect either a higher affinity for gastrin of the antiserum compared with ligandin, or may represent recognition of different sites of the gastrin molecule by these two proteins. Nevertheless, further studies with purified ligandin would be required to determine the kinetics of this interaction. The failure of liver supernate to significantly bind ^{125}I itself excludes the possibility that we were dealing with iodine and not gastrin binding to ligandin, and this specificity is further shown by the absence of glucagon or insulin binding to the transport protein. The binding of gastrin to ligandin may well be related to the highly acidic nature of the gastrin molecules with its large number of charged glutamic acid residues. Other studies using synthetic peptides lacking these residues should resolve this question.

Ligandin, the major organic anion binding protein in liver, kidney and proximal small bowel, has been shown to be important in uptake of drugs, dyes, cholecystographic agents, hormones and carcinogens when given in vivo or when added in vitro to supernatant fractions prepared from homogenates of these tissues [6–8]. Phylogenetic [14] and ontogenetic [15] studies reveal correlation between appearance of ligandin in liver and the ability to transfer organic anions from plasma into the liver. Binding of gastrin to ligandin may explain the uptake of this hormone at high delivery rates by the liver [16]. The findings that gastrin binds in vitro to ligandin may indicate a functional role of the latter protein in the uptake of this hormone by the liver [16] and correlates with the observation that gastrin is concentrated in the kidney and gut [3–5].

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References

[1] Strauss, E. and Yalow, R. S. (1974) Gastroenterology 66, 936–943.
[2] Walshe, J. H., Debas, H. T. and Grossman, M. I. (1973) Gastroenterology 64, 723 (Abstract).
[3] Chan Yip, B. S. S. and Jordan, P. H. (1973) Surgery 74, 412–419.
[4] Evans, J. C. W., Rheeder, D. D., Becker, H. D. and Thompson, J. C. (1974) Gut 15, 112–115.
[5] Newton, W. T. and Jaffe, B. M. (1971) Surgery 69, 34–40.
[6] Levi, A. J., Gatmaitan, Z. and Arias, I. M. (1969) J. Clin. Invest. 48, 2156–2167.
[7] Fleischner, G., Mishkin, S., Reyes, H., Robbins, J., Levi, A. J., Gatmaitan, Z. and Arias, I. M. (1971) J. Clin. Invest. 50, 31.
[8] Litwack, G., Ketterer, B. and Arias, I. M. (1971) Nature 234, 466–467.
[9] Ganguli, P. C. and Hunter, W. N. (1970) in: Iodination of gastrin in Radioimmunoassay Methods, pp. 54–60. (Kirkham, K. E., Hunter, W. M., E. & S. Livingstone, eds.).
[10] McFarlane, A. S. (1958) Nature 182, 53.
[11] Vinik, A. I. and Grant, B. J. (submitted for publication).
[12] Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51, 660–672.
[13] Kirsch, R. E., Kamisaka, K., Fleischner, G. and Arais, I. M. (submitted for publication).
[14] Levine, R. J., Reyes, H. and Levi, A. J. (1971) Nature 231, 277–280.
[15] Levi, A. J., Gatmaitan, Z. and Arias, I. M. (1970) New Eng. J. Med. 283, 1136–1139.
[16] Vinik, A. I., Grant, B. J., Hickman, R. and Terblanche, J. (submitted for publication).