GUT GLUCAGON: A COMMON RECEPTOR SITE WITH PANCREATIC GLUCAGON IN LIVER CELL PLASMA MEMBRANES

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1. Introduction

Gut extracts that contain glucagon-like immunoreactivity (GLI) have been shown to possess glycogenolytic [1] and insulinogenic [2] activity. It is, however, unknown whether these extracts exert their effects through the same receptor site as pancreatic glucagon in glucagon-sensitive target tissues, although a recent report suggests that GLI-containing extracts from porcine gut interact with pancreatic glucagon receptors in β cell membranes [3]. We found that GLI-containing fractions prepared from porcine gut interact with the pancreatic glucagon receptors and stimulate the adenylate cyclase in liver plasma membranes, indicating that a common receptor site is involved in the interaction of pancreatic and gut glucagon with the liver. Furthermore, our data indicate the existence of a specific receptor site for the pancreatic glucagon.

2. Materials and methods

Porcine jejuno-ileons were resected immediately after slaughter. Extracts of the mucosa obtained by a modification (see legend to fig. 1) of the Kenny procedure [4] were fractionated on Bio Gel P 10 as indicated in fig. 1.

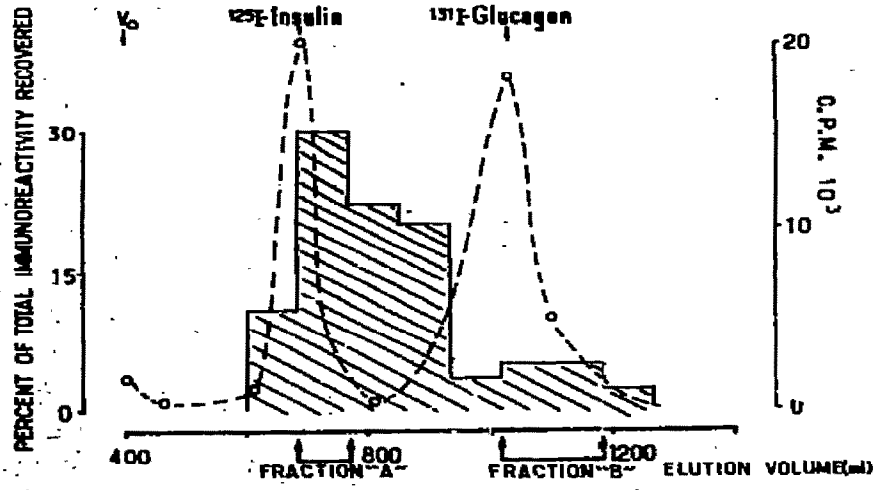

Fig. 1. Gel filtration of porcine gut extracts on a 5 × 100 cm column of Bio Gel P 10 (100–200 mesh, Bio Rad) that was equilibrated and eluted with 0.05 M NH_4HCO_3. The percentage of total GLI recovered (dashed area) is plotted as a function of the elution volume. Radioactivity (dotted lines) refers to [^{125}I]insulin and [^{131}I]pancreatic glucagon used in tracer amounts as markers. The material that was submitted to gel filtration consisted of proteins precipitated by 10% CCl_3COOH and obtained after delipidation of acid–alcohol extracts [4] from jejuno-ileal mucosa.

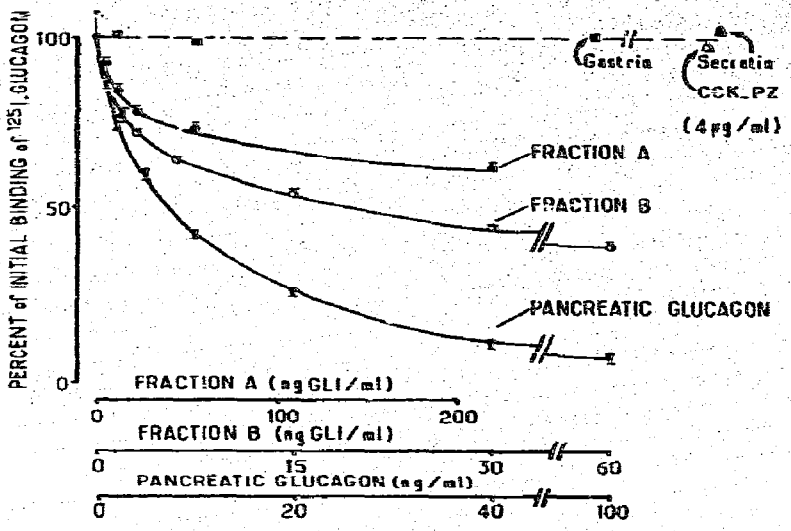

Fig. 2. Displacement of [^{125}I]pancreatic glucagon from its liver receptors by unlabeled pancreatic glucagon and by fractions A and B (see fig. 1) of gut glucagon. Membranes (0.2–0.3 mg protein/ml incubation medium) were incubated for 30 min at 30°, pH 7.5, in Krebs Ringer phosphate buffer containing 10 mM Tris, 2.4% (w/v) bovine serum albumin (Fraction V, Pentex), 2 000 U of Kallikrein inhibitor (Zymofren, Specia)/ml as an inhibitor of glucagon degradation, 20–30 pM of [^{125}I]pancreatic glucagon and various amounts of unlabeled peptides as indicated. The membrane-bound [^{125}I]glucagon was isolated as previously described [11, 12]. The initial binding of [^{125}I]glucagon is the percentage of the total radioactivity bound to the membranes in the absence of unlabeled hormone. This percentage was about 30%. Before use, the lyophilized chromatographic fractions had been dissolved in 25 mM Tris-HCl, pH 7.5, and dialyzed overnight against the same buffer.

The protein contents were assayed by the Lowry method [5]. GLI was evaluated in each lyophilized fraction by glucagon radioimmunoassay [6, 7] using antisera that cross react with gut GLI; similar results were obtained with three such antisera. In all of four fractionations of the jejuno-ileal extracts, the distribution of the GLI conformed to the pattern depicted in fig. 1: GLI was recovered as a major peak with a maximum close to the insulin marker (fraction A) and the remaining immunoreactivity was eluted almost coincidently with the pancreatic glucagon marker (fraction B).

Purified plasma membranes (step 11, [8]) were prepared from rat livers according to Neville [8]). Pork pancreatic mono [^{125}I]glucagon was prepared as described previously [9] using a similar method to that employed for the preparation of monoiodoinsulin [10]. Experiments measuring the binding of glucagon to its specific receptors in liver plasma membranes were performed as described elsewhere [11, 12] with the modifications indicated in the legend to fig. 2. This method makes it possible to measure glucagon–receptor interaction at concentrations of the hormone as low as 0.01–1.0 nM. The adenylate cyclase activity was measured by radioimmunoassay of cyclic AMP [13]. This method permits the specific measurement of the cyclic AMP produced in quantities as low as 1 pmole.

3. Results and discussion

Both fractions A and B inhibited the specific binding of [^{125}I]pancreatic glucagon to liver membranes (fig. 2). In sharp contrast, large amounts of synthetic gastrin* as well as highly purified secretin† and cholecystokinin-pancreozymin† (CCK-PZ) failed to interfere with the binding of pancreatic glucagon (fig. 2). Similarly, no significant effect was observed with the vasoactive intestinal polypeptide (VIP) [14]. Small amounts of fraction B, on the basis of their GLI content, were almost as effective as equivalent amounts of unlabeled pancreatic glucagon in displacing the [^{125}I]pancreatic glucagon from its receptors. Fraction A also interfered with the binding of pancreatic

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† Donated by Drs. V. Mutt and J.E. Jorpes, Stockholm, Sweden.

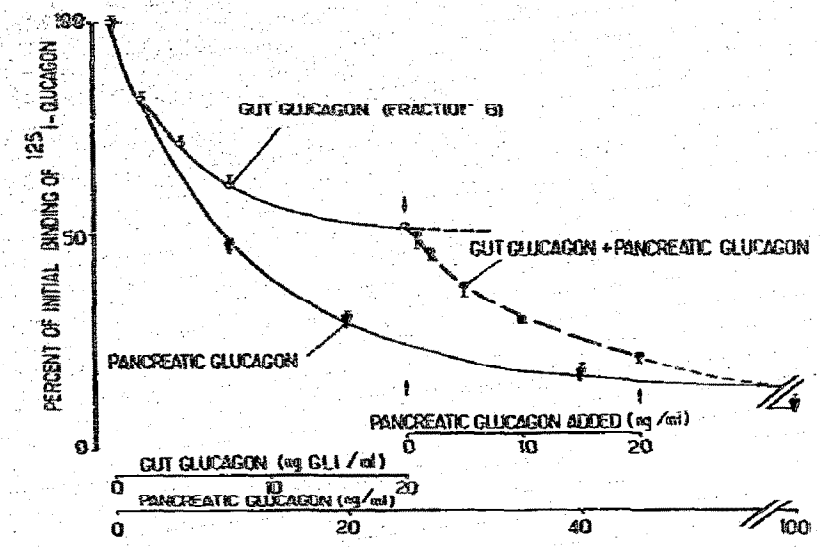

Fig. 3. Displacement of [^{125}I]pancreatic glucagon from its specific receptor by unlabeled pancreatic glucagon. [^{125}I]pancreatic glucagon was incubated with membranes in the absence and in the presence of increasing concentrations of unlabeled pancreatic or gut (fraction B) glucagon. Tubes containing a saturating concentration of gut glucagon for the common receptor site (see also fig. 2) were incubated in the absence and in the presence of unlabeled pancreatic glucagon added (dashed line and solid circles between the 2 arrows). Other conditions were as described in the legend to fig. 2.

glucagon but to a much lesser extent than fraction B on the basis of their respective GLI contents. The displacement of [^{125}I]pancreatic glucagon by relatively small amounts of these fractions indicates the presence of a glucagon-like material that shares a common receptor site with the pancreatic glucagon, and suggests that this material possesses a structural analogy with the pancreatic glucagon molecule.

Larger amounts of fractions A and B failed to displace further the pancreatic glucagon in direct proportion to their GLI contents (fig. 2). This failure to obtain a complete displacement of the pancreatic glucagon by larger amounts of both fractions suggests that the glucagon receptor population is heterogeneous and that a site that is specific for the pancreatic glucagon does not interact with the gut glucagon-like material. The existence of a specific receptor site for the pancreatic glucagon is demonstrated by the following observation. In the presence of a concentration of gut glucagon that saturates the common site, low concentrations of unlabeled pancreatic glucagon are capable of displacing completely the remainder of the bound [^{125}I]pancreatic glucagon (fig. 3).

Both fractions A and B also stimulated adenylate cyclase in liver plasma membranes (fig. 4). Gastrin, secretin and CCK-PZ were ineffective in stimulating the adenylate cyclase. As was observed in the binding experiments, fraction B was much more effective than fraction A at equivalent GLI concentrations, and small amounts of fraction B did increase the adenylate cyclase activity whereas larger amounts were relatively ineffective in increasing further the level of stimulation (fig. 4).

Table 1 summarizes the effect of fractions A and B in liver plasma membranes. At low concentration of fraction B there is a good agreement between its GLI content and its efficacy to displace the labeled pancreatic glucagon. Fraction B, which elutes almost coincidently with the pancreatic glucagon marker, is much more effective than fraction A which contains the bulk of the glucagon-like immunoreactivity. Both fractions, at all concentrations tested, are relatively less effective in stimulating the adenylate cyclase than in displacing the [^{125}I]pancreatic glucagon.

Our findings provide additional support to the possible implication of a glucagon-like material in the glycogenolytic effect of gut extracts that had been observed previously [15] and which was described with the peak II of GLI after gel filtration of canine gut extracts [1]. Possible contaminants such as secretin, gastrin, CCK-PZ and/or VIP, would not account for

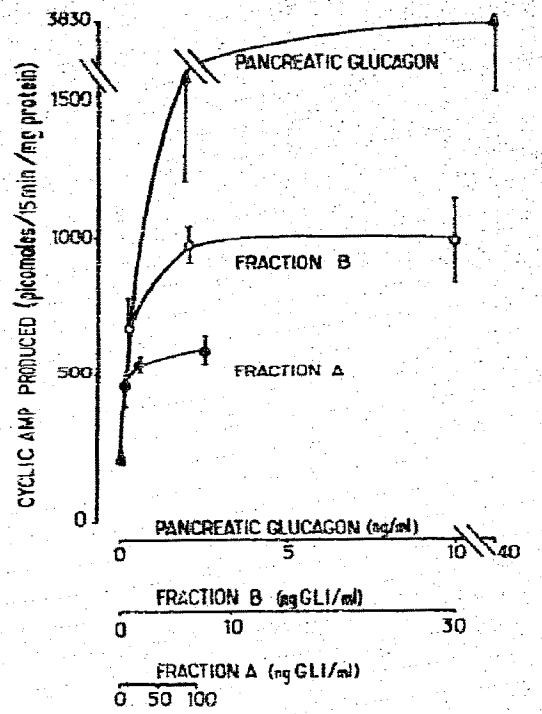

Fig. 4. Stimulation of adenylate cyclase by pancreatic glucagon and by fractions A and B in liver plasma membranes. The incubation solution contained, in a 250 µl final volume, 0.8 mM ATP, 20 mM creatine phosphate, 1 mg/ml phosphocreatine kinase, 2.5 mM theophylline, 5 mM $MgCl_2$, 20 mM Tris-HCl (pH 7.5), 0.4% (w/v) bovine serum albumin, 1 mM EDTA and 100 µg/ml bacitracin. Reactions were started in the absence or in the presence of hormone(s) as indicated, with addition of enzyme at about 50 µg of membrane protein, i.e. 0.2 mg/ml final conc. Incubations were performed for 15 min at 30°. The cyclic AMP produced was measured by radioimmunoassay [13]. Each point is the mean ± S.E. of triplicate determinations.

the effects observed with our fractions since these hormones were ineffective in displacing the [^{125}I]-glucagon as well as in stimulating the adenylate cyclase.

In vitro studies with direct measurement of glucagon–receptor interactions such as those reported here appear to be very important in investigating further the biochemical relationships between the pancreatic and the gut glucagons.

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Table 1
Effects of gut glucagon in liver membranes.

	Pancreatic glucagon equivalents (ng)		
	GLI content	Displacement of [^{125}I]glucagon from receptors	Activation of adenylate cyclase
	4.4	0.5	0.2
Fraction A	22	1.5	0.3
	110	3.0	0.35
	1.2	1.0	0.4
Fraction B	6	4.0	0.8
	30	8.0	0.8

From data shown in figs. 2 and 3, equivalences (in ng of pancreatic glucagon) were determined for both fractions of gut glucagon by comparing their ability in displacing [^{125}I]pancreatic glucagon from receptors (center column) and in stimulating adenylate cyclase (right column), to the ability of pancreatic glucagon. The left column indicates the corresponding GLI content of each fraction.

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