A Radioreceptor-Assay for Glucagon: Binding of Enteroglucagon to Liver Plasma Membranes

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Summary. A radioreceptor-assay for glucagon was developed employing pig liver plasma membranes isolated by means of an aqueous two-phase polymer system. The assay is simple, precise, and has a detection limit of 40 pg/ml. Acid-ethanol extracts of porcine intestinal mucosa and enteroglucagon purified by affinity chromatography interfered strongly with the binding of ^{125}I-labelled glucagon. The affinity of enteroglucagon for the membranes was lower than that of glucagon, but even physiological concentrations of the former interfere with glucagon binding, indicating that enteroglucagon may compete with pancreatic glucagon for binding to the hepatocyte under physiological conditions.

Key words: Glucagon, affinity chromatography, hormone receptor, radioligand assay, gastrointestinal hormones, radioimmunoassay.

The glucagon receptor of the plasma membranes of rat liver has been extensively studied (see recent reviews by Rodbell [1], Birnbaumer [2], and Rodbell [3]). It is, however, as yet unclear whether enteroglucagon[1] (see Moody [4] for recent review) interacts with the hepatic receptors [5—9]. In the present study the development of a radioreceptorassay [10] for glucagon and the interaction of different preparations of enteroglucagon with the glucagon receptor are described.

Materials and Methods

Membrane Preparation

Pig liver tissue was obtained by hepatectomy on anesthetized 30—40 kg Danish pigs. Plasma membranes were isolated using the aqueous two-phase polymer system of Brunette and Till [11], as described by Lesko et al. [12]. The high content of connective tissue in porcine liver made special treatment necessary and the following procedure was adopted. Livers were cut out of anesthetized pigs as quickly as possible and perfused via the portal vein with at least 2 liters of ice-cold physiological saline. When the major part of the liver had blanched, approximately 300 g of tissue were cut out of the blanched parts, minced roughly with scissors, and homogenized very briefly in a Waring type blender (Ballerup Senior Mixer, Copenhagen, Denmark, 30 sec at position 1)

in the presence of 4 vol of ice-cold medium, according to Ray [13], i.e. 1 mM $NaHCO_3$ (pH 7.5) containing in addition 0.5 mM $CaCl_2$. The homogenate was transferred to a sieve (pore size 1.5 mm) and strained with a ladle. This step was repeated with a finer sieve (0.9 mm). These procedures removed large amounts of connective tissue. The resulting mash (100 g) was diluted with 3 volumes of medium and homogenized with a Dounce homogenizer. The homogenate was diluted to 1.2 liters with medium and centrifuged in the PR_2 (IEC) refrigerated centrifuge, according to Ray [13], in order to obtain the membrane-rich, low speed pellet.

The polymers were prepared as described by Lesko; it was found, however, that more than 48 hrs were needed to ensure complete partition of the two phases. 96 hrs were found to be satisfactory. The pellet was suspended in 80 ml of the upper phase, 10 ml aliquots were pipetted into 8 50 ml centrifuge tubes, to which were added 10 ml of the lower phase solution. After mixing, the tubes were spun for 15 min at $2000 \times G$ in a refrigerated centrifuge (Hettich), and the resulting precipitate suspended in another 80 ml of upper phase, divided into eight parts etc., according to Lesko. The material from the interphase was finally washed thoroughly in Krebs-Ringer bicarbonate buffer and stored either frozen in dry ice or freeze-dried at —20° C. The whole procedure could be completed in less than 7 hours.

[125]I-Labelled Glucagon

Monoiodinated, monocomponent ^{125}I-labelled glucagon, prepared according to Jørgensen and Larsen [14], was a gift from U. D. Larsen, the Novo

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[1] In this report enteroglucagon is defined as a substance (or substances), which is extractable from the gastrointestinal mucosa by techniques that would also extract and purify pancreatic glucagon, and which binds to certain crossreacting antiglucagon antibodies.

Research Institute, Copenhagen. Specific activity was from $100-200$ μCi/μg, with an average of 160 μCi/μg.

Glucagon and Enteroglucagon

Glucagon for standards was Monocomponent Glucagon, a gift from L. G. Heding, the Novo Research Institute, Copenhagen. A crude acid-ethanol extract of porcine intestinal mucosa (MUC 101), prepared according to Moody et al. [15], was a gift from F. Sundby, the Novo Research Institute. Furthermore, enteroglucagon was extracted from porcine intestinal mucosa according to Kenny and Say [16], as described by Murphy et al. [6], with the exception that mucosal tissue was obtained by scraping off the luminal surface of the total small intestine, excised as quickly as possible from an anesthetized pig, and frozen immediately afterwards between blocks of dry ice. After extraction the material was freeze-dried.

A part of the extract corresponding to 100 g of porcine intestinal mucosa was purified by affinity chromatography [17]. 20 ml of anti-glucagon-immunosorbent was packed in a 20×2 cm column and equilibrated with 0.05 M sodium-phosphate buffer, pH 7.5, containing in addition 0.15 M NaCl. The capacity of the column was determined by the application of 10 mg of glucagon (NOVO), dissolved in 10 ml 0.4 M sodium-phosphate buffer, pH 7.5, containing in addition a trace amount of labelled glucagon. After extensive washing with the equilibration buffer, bound material was eluted by means of 1 M ammonia. The UV-absorption of the column effluent was monitored continuously at 253 nm (Uvicord, LKB) and all fractions were analysed for radioactivity. The freeze-dried Kenny-extract was dissolved in 0.4 M sodium-phosphate buffer, pH 7.5, and applied to the column. After elution of unbound material, the column was washed extensively with 0.125 M ammonium-bicarbonate and finally eluted with 1 M ammonia, which yielded a higher recovery of glucagon from the column compared to acid ethanol, formic acid, dioxane, HCl, urea, and Guanidine-HCl. The eluted fractions were freeze-dried for subsequent radio-immunoassay, protein determination, and receptor-assay.

Immunosorbent

10 ml of antiserum 4304 [18], which binds enteroglucagon and pancreatic glucagon with the same energy, and which is routinely diluted 100 000 times for assay use, was precipitated with sodium-sulphate according to Wide [19]. The precipitate was dissolved in 30 ml phosphate buffer, 0.05 M, NaCl 0.5 M, pH

6.5, to which was added 35 ml CNBr-activated Sepharose 4 B® (Pharmacia, Uppsala) freshly prepared according to the manufacturer's instructions. The mixture was allowed to react overnight, treated with 1 M Glycine at pH 8.5 for 2 hrs and washed extensively with buffers of high ionic strength and varying pH as advised by the manufacturer. The immunosorbent was tested in a solid-phase radioimmunoassay system, a modification of a previously described technique [20], and enteroglucagon (MUC 101) and pancreatic glucagon were still bound with identical affinity. The immunosorbent was stored at 4° C, and was found to be stable for more than six months.

Radioimmunoassay

Radioimmunoassay [18] was performed using an ethanol precipitation technique [21]. The antiserum used, 4304 [18], is supposed to measure enteroglucagon quantitatively against standards of pancreatic glucagon, because dilution curves of pancreatic glucagon and extracts of intestinal mucosa (MUC 101) are superimposable over any concentration range. The detection limit is 15 pg/ml and the within-assay coefficient of variation is 0.05 in the working range $(25-500$ pg/ml). During oral glucose tolerance tests, marked increases in total glucagon concentration are found with this antiserum.

Radioreceptor-Assay

Standards or samples were made up in Krebs-Ringer bicarbonate buffer, containing in addition 1% (w/v) Gelatine. For evaluation of non-specific binding a standard of 200 ng/ml was included in all experiments. The buffer was gassed with an oxygen-carbon dioxide mixture (96%/4%) and kept in a bath at 37° C. pH was 7.4. 2 ml of standard or sample were mixed with 200 μg of membranes[2], which were suspended in distilled water immediately before use. The tubes were gently rotated for 30 min at room temperature. Then 100 pg ^{125}I-labelled glucagon was added to the mixture, and incubation was continued for 15 minutes. Separation was performed either by Millipore filtration (Swinnex® filter holders and EGWP filters) or by centrifugation at $3000 \times G$ for 10 min followed by 3 washes with incubation buffer. Filter discs or precipitates were counted in an automatic gamma scintillator (Selektronik, Denmark). All samples and standards were assayed in duplicate.

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[2] Protein analysis according to Lowry et al. [22]; before analysis the membrane suspension and albumin standards were heated in equal volumes of 1 N NaOH until completely dissolved.

Specificity of the receptor assay was examined by incubation of membranes with high concentrations of gastrin (synthetic human gastrin-17, obtained from ICI, Cheshire, England), secretin (purified natural porcine secretin, a gift from V. Mutt, GIH-laboratory, Stockholm Sweden), Vasoactive Intestinal Polypeptide (a gift from E. K. Frandsen, the Novo Research Institute, who received the peptide as a gift from S. I. Said, Veterans Administration Hospital, University of Texas), insulin (highly purified porcine insulin, Monocomponent insulin, a gift from L. G. Heding, the Novo Research Institute), and cholecystokinin (10% pure, from the GIH-laboratory, Stockholm, Sweden). Polyacrylamide gel-electrophoresis was performed by means of a Quickfit apparatus as a modification of System 3, according to Maurer [23]. Gels were 20% acrylamide in Tris-HCl buffer at pH 8.9, which contained in addition 8 M Urea (analytical grade); compartment buffer was Tris-Glycine at pH 8.3. Samples were applied in 8 M Urea, 10% sucrose, and bromophenolblue added as a marker. Electrophoresis was performed at 2 mAmp/tube until the colour marker had reached the bottom of the tube. The gels were cut into sections of 2 mm, which were eluted overnight with radioimmunoassay buffer [18], followed by radioimmunoassay. Other tubes were stained with amido black. Labelled and unlabelled glucagon was applied to control-tubes. These were either counted or assayed for glucagon content.

Results

By means of the aqueous two-phase polymer system large amounts of membrane material were isolated from pig liver; 200 — 300 mg of membrane protein [22] were obtained in each of 5 experiments. From initial experiments, in which labelled hormone alone or labelled hormone plus excess unlabelled hormone were incubated with increasing amounts of membrane, it appeared that the membranes did bind glucagon specifically (the binding was displaceable by excess unlabelled hormone) and that binding increased proportionally with the amount of membrane present. For all further experiments 200 μg of membrane-protein were added per incubation; this was sufficient to bind 10 — 14% of the labelled hormone. Nonspecific binding (radioactivity in precipitates, which was not displaced by excess unlabelled hormone) constituted an average of 8% of total binding and never exceeded 10%. A study of the time course of binding (Fig. 1) suggested that equilibrium was reached within 30 min at room temperature. As dissociation of glucagon (not shown) is very slow under
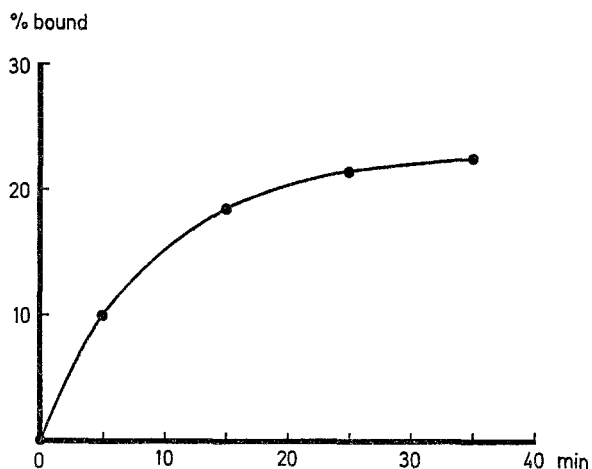

Fig. 1. Time course of binding of ^{125}I-labelled glucagon to pig liver plasma membranes. 200 μg membrane protein and 40 pg labelled glucagon in a volume of 2 ml buffer (see text) were incubated at 22° C. Separation was performed after increasing incubation periods as indicated on the abscissa. Ordinate: per cent of labelled hormone specifically bound to membranes
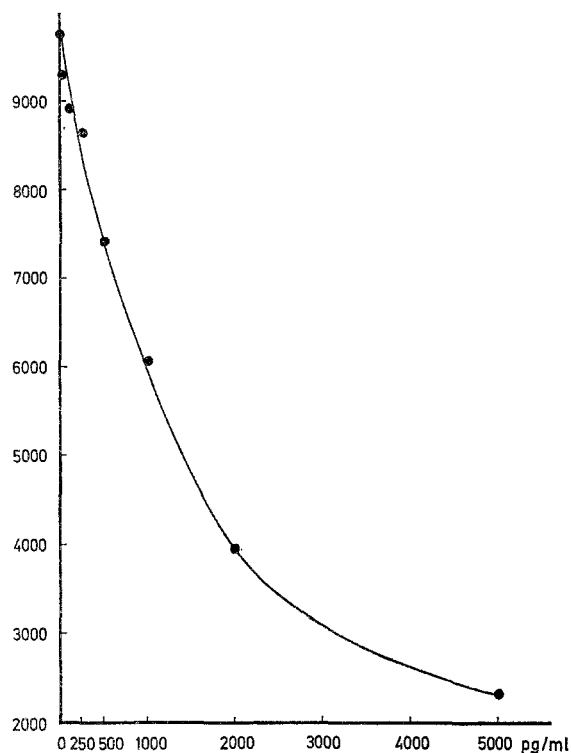

Fig. 2. Standard curve obtained with porcine liver cell membranes, isolated according to Lesko [12]. Incubation conditions as described in the methods section

these conditions [3], standards or samples and receptor were incubated for 30 min before the addition of a relatively large amount of labelled hormone (100 pg) for a relatively short period (15 min) before separation. The resulting standard curve is shown in

Fig. 2. The detection limit of the receptor-assay (the smallest amount that can be discriminated significantly from zero) was 40 pg/ml. Sixfold assays of samples in the low and high range yielded coefficients of variation of 0.06 — 0.07.
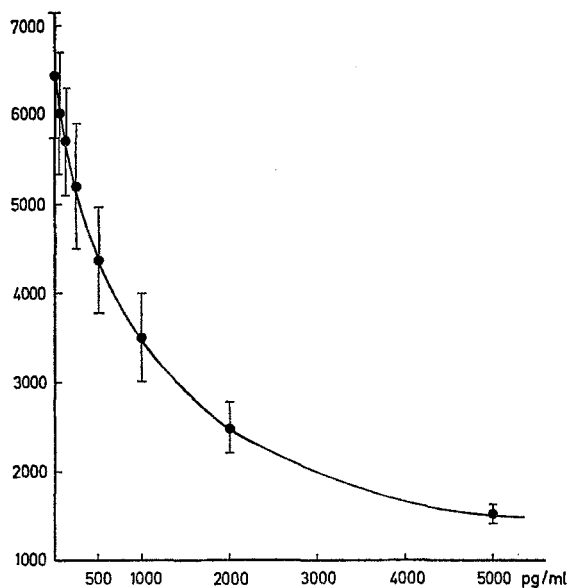
The binding capacity of the membranes and the equilibrium constant, K, for the hormone-receptor reaction were calculated by Scatchard analysis [24] of the data of Fig. 2. A straight line was the best fit to the results (Fig. 4). Hence the binding capacity was

Fig. 3. Reproducibility of standard curves. Means (± SEM) of the values from six consecutive standard curves. Incubation conditions as described in the methods sections. Ordinate: counts in precipitate. Abscissa: concentration of glucagon
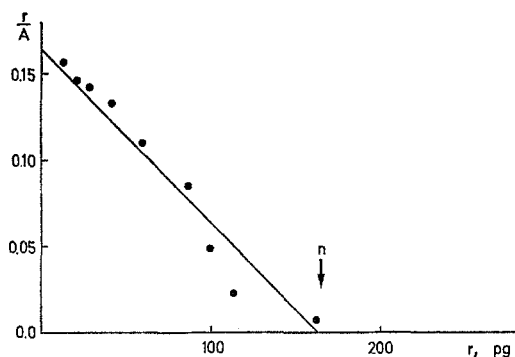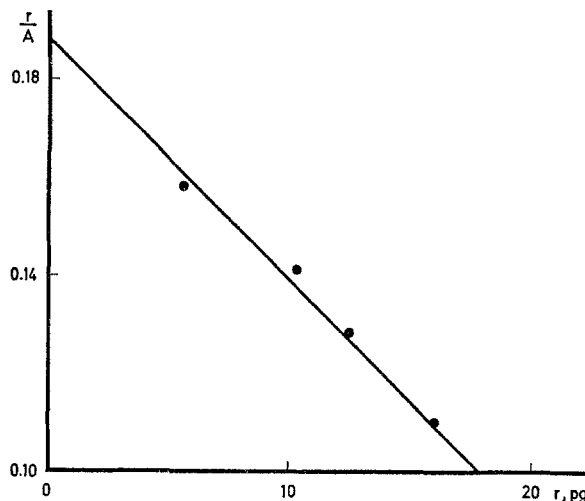

Fig. 5. Binding of ^{125}I-labelled glucagon to liver cell plasma membranes. Increasing amounts of labelled glucagon were incubated with membranes for 45 min (specific activity 200 μCi/μg). The binding data were transformed to fit the equation r/A = K (n-r), where A is the concentration of unbound hormone, r the amount of receptor-bound hormone, n the maximum binding capacity, and K the equilibrium constant; a plot was constructed of r/a versus r according to Scatchard [24]

Fig. 4. Scatchard plot [24] of the data from Fig. 2, transformed to fit the equation r/A = K (n-r), where A is the concentration of unbound hormone, r the amount of receptor-bound hormone, n the maximum binding capacity, and K the equilibrium constant
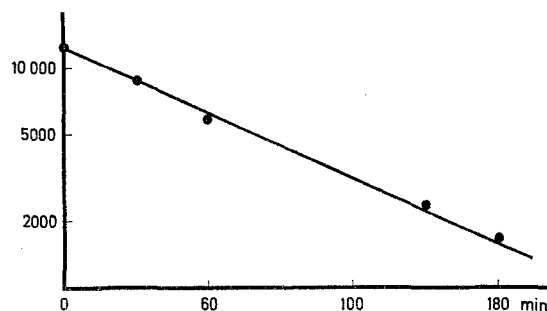

Fig. 6. Degradation of membranes at room temperature. Membranes were suspended in incubation buffer, and labelled hormone was added for 15 minutes at the times indicated on the abscissa. Separation was performed by centrifugation as described in the methods section. The bound radioactivity is plotted against preincubation time. Ordinate: counts in precipitate (note logarithmic scale)

The means (± SEM) of the values from 6 consecutive standard curves are shown in Fig. 3. The variation is largely due to variations in the quality of the labelled hormone, the binding activity of which decreased upon storage, which was also demonstrated in the radioimmunoassay.

163 pg/200 μg membranes or 0.5 pmol/mg membrane protein, and K = 7.0 × 10⁹ l/mol. The equilibrium constant for the binding of labelled glucagon to liver cell membranes was determined from Scatchard analysis [24] of data from an experiment in which increasing amounts of labelled hormone were incubated

with membranes for 45 minutes, i.e. the total incubation time of the assay (Fig. 5). K was now 7.0×10^{10} l/mol.

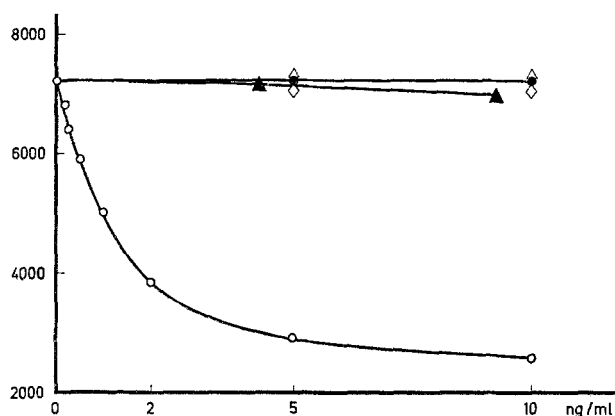The membranes were found to retain binding activity for more than 6 months when stored at $-60°$ C,

Fig. 7. Specificity of the receptor assay. Membranes were incubated with various concentrations of glucagon (○), insulin (▲), gastrin (△), secretin (◇) or Vasoactive Intestinal Polypeptide (●). Incubation conditions as described in the methods section. Ordinate: ^{125}I-labelled glucagon bound to membranes (counts in precipitate). Abscissa: concentration of peptides
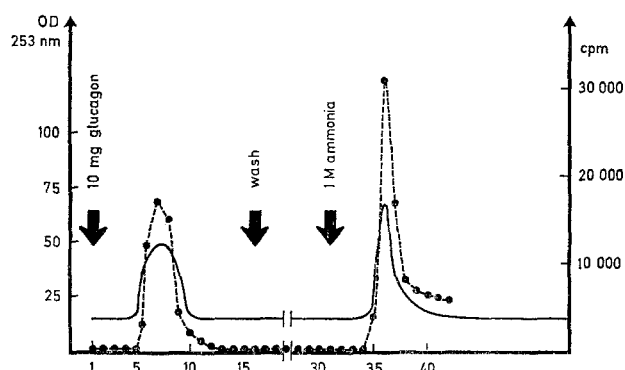

Fig. 8. Capacity of anti-glucagon immunosorbent column. 10 mg glucagon (NOVO) in 10 ml 0.4 M sodium phosphate buffer, pH 7.5, containing in addition a trace amount of ^{125}I-labelled glucagon, was applied on an anti-glucagon column (see text), which was equilibrated with 0.05 M sodium phosphate buffer, pH 7.5, containing in addition 0.15 M NaCl. Flow was not interrupted. After the breakthrough (fractions 5—12) the column was washed extensively with equilibration buffer. The bound glucagon was subsequently eluted with 1 M ammonia. Left column: readings (———) in arbitrary units from Uvicord (LKB), right column: radioactivity (·····) in collected fractions (3 ml) of the column effluent (abscissa)

The rather rapid loss of binding capacity at $0°$ C prompted an investigation of the degradation of membranes in suspension in assay buffer at room temperature (Fig. 6). A monoexponential loss of capacity with
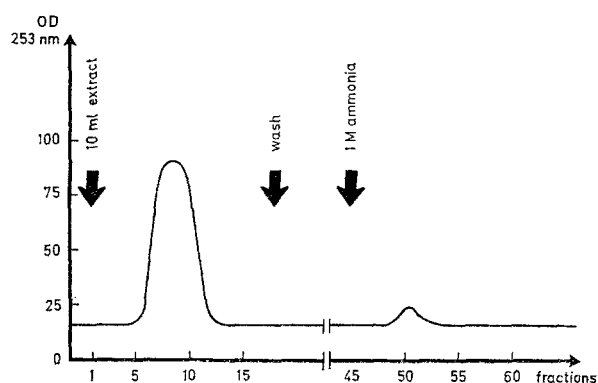

Fig. 9. Affinity chromatography of porcine intestinal mucosa extracted according to Kenny [16]. Freeze-dried extract of 100 g mucosa dissolved in 10 ml 0.4 M sodium phosphate, pH 7.5, was applied to an antiglucagon column, which was equilibrated with 0.05 M sodium phosphate buffer, pH 7.5, containing in addition 0.15 M NaCl. After the breakthrough (fractions 5—12) the column was washed extensively with 0.125 M ammonium-bicarbonate, and bound material was subsequently eluted with 1 M ammonia. Abscissa: collected fractions (3 ml) of column effluent. Column at left: readings in arbitrary units from a Uvicord (LKB)
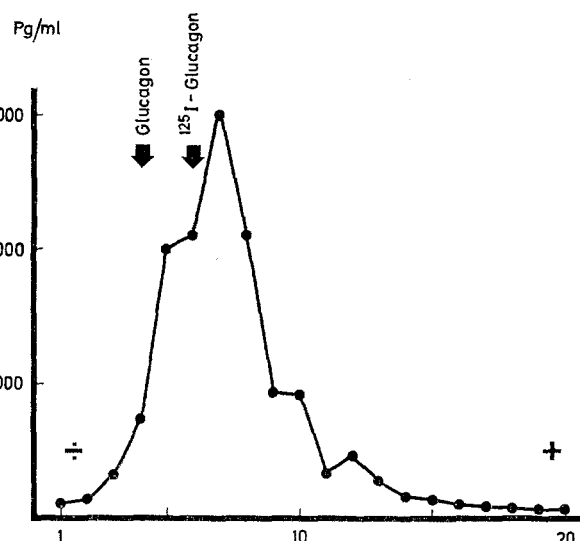

Fig. 10. Polyacrylamide gel-electrophoresis of purified enteroglucagon. Concentration of enteroglucagon in 2 mm sections of gel eluted with 2 ml buffer for radioimmunoassay. Abscissa: number of section. Ordinate: concentration of enteroglucagon

and for more than 5 months when freeze-dried and stored at $-20°$ C. With membranes in suspension at $0°$ C binding capacity was halved in 7 hrs and reduced to 10% after 20 hrs. Refreezing also destroyed binding activity.

a half life of 60 min was found. Because of the instability of the membranes only small series of incubations were performed at a time, and all manipulations were performed simultaneously or as fast as possible.

The results of the specificity-analysis are shown in Fig. 7. None of the peptides tested were able to inhibit binding of labelled glucagon. When cholecystokinin was assayed in a calculated concentration of 100 ng of cholecystokinin/ml, a crossreaction of 0.8% was noted. This preparation contained 2 ng/ml of glucagon-like immunoreactivity, as determined by radioimmunoassay.

fractions (47 — 66) were freeze-dried; a total of 1 mg of protein was eluted. In contrast with the data obtained with MUC 101, neither Kenny extracts nor the purified material yielded dilution-curves superimposable on dilutions of pancreatic glucagon when subjected to radioimmunoassay. It is therefore not possible to calculate the enteroglucagon content of these preparations. Estimates from dilutions within the
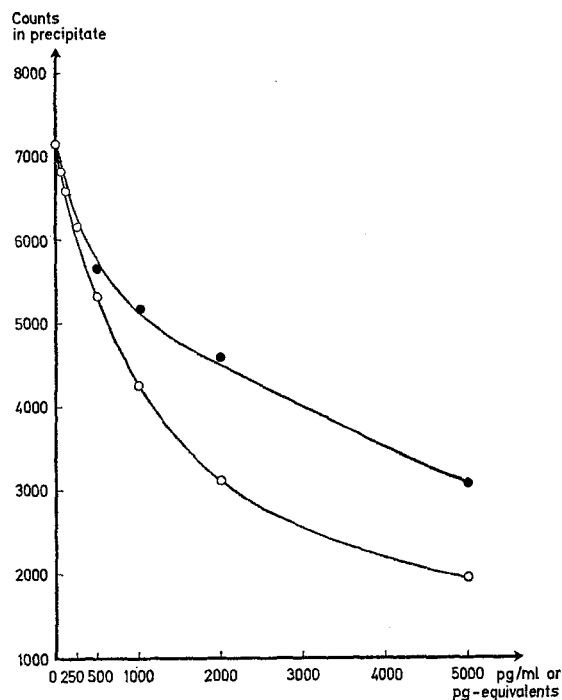

Fig. 11. Binding of enteroglucagon (MUC 101) to pig liver plasma membranes. Dilutions of pancreatic glucagon and enteroglucagon are compared. Incubation conditions as described in the methods section. Ordinate: counts in precipitate. Abscissa: concentrations of glucagon (open circles) and concentrations of enteroglucagon (closed circles) as measured by radioimmunoassay [18] against standards of glucagon
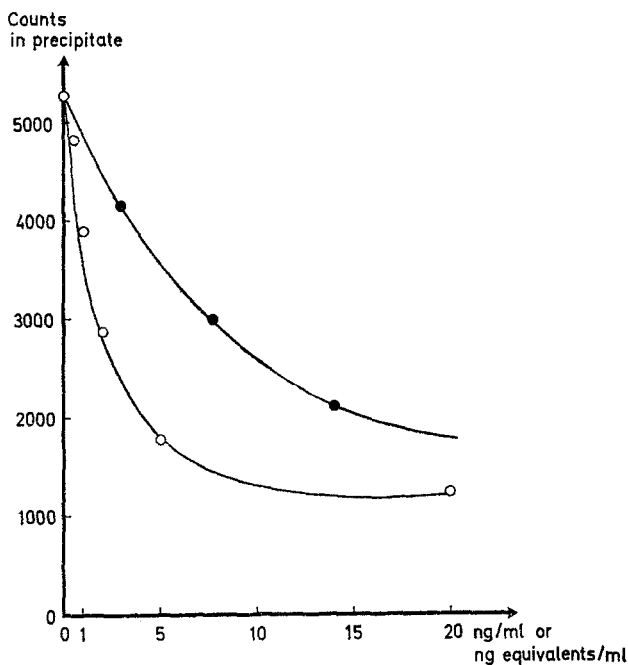
Fig. 12. Binding of enteroglucagon purified by affinity chromatography to pig liver plasma membranes. Dilutions of pancreatic glucagon (open circles) and enteroglucagon (closed circles) are compared. Incubation conditions as described in methods section. Ordinate: counts in precipitate. Abscissa: concentrations of glucagon and apparent concentrations of enteroglucagon as measured by radioimmunoassay [18] against standards of glucagon

Enteroglucagon

MUC 101 was found to contain 600 ng equivalents gut glucagon/mg. This value was found also with a different antiserum [21].

The results of the affinity chromatography can be seen in Figs. 8 and 9. The capacity of the column was 5 mg of pancreatic glucagon. Recovery of radioactivity was 96%. As expected a large amount of UVabsorbing material, which did not contain glucagon immunoreactivity, passed the column unretarded when Kenny extract was applied (Fig. 9). Enteroglucagon was eluted subsequently in sufficient amounts to be detected by the Uvicord. The collected

working range of the assay, however, suggest a purity of 10 μg weight equivalents of pancreatic glucagon/ mg protein, which means a purification of 100 times by affinity chromatography.

When subjected to polyacrylamide gel-electrophoresis, the bulk of the purified enteroglucagon eluted as a single peak close to the position of labelled glucagon; both labelled and unlabelled glucagon were located in sharp bands (1 mm), contrasting with the width of the enteroglucagon peak (Fig. 10).

No bands were seen upon staining with amido black.

When enteroglucagon, regardless of preparation, was incubated with membranes, it prevented the

binding of labelled glucagon to the receptor in a concentration-dependent manner. The inhibition curves were never parallel to the standard curves. Dilution curves of MUC 101 and purified enteroglucagon are shown in Figs. 11 and 12; the concentrations of immunoreactive material are shown on the abscissa.

The apparent affinity of enteroglucagon to the receptors is lower than that of glucagon; 2.4 (Fig. 11) and 4.6 (Fig. 12) times higher concentrations of enteroglucagon are needed to decrease the binding of labelled glucagon by 50%. These dilution curves resemble those obtained with usual antisera against glucagon [21]. Concentrations of enteroglucagon lower than 500 pg equivalents/ml significantly interfere with binding of the labelled glucagon (Fig. 11).

Discussion

In the search for a suitable receptor-preparation for the development of a glucagon-radioreceptor-assay, liver cell plasma membranes were chosen, because the liver appears to be the most important target organ for the physiological action of glucagon [25], and because glucagon binding sites of high affinity were described for rat liver plasma membranes [1]. Secondly, pig livers were chosen, because it was intended to investigate the behaviour of porcine enteroglucagon in the receptor-assay. Thirdly, the method of Brunette and Till [11], as described by Lesko [12], was chosen for the isolation of plasma membranes because of its simplicity and the reported high yield and purity of the product. Isolation of plasma membranes from pig liver has been described by Lutz and Frimmer [26]; their method of isolation, however, is quite complicated and takes more than 24 hrs, which is less desirable when recovery of biological activity is intended. The few modifications of the homogenization procedure described made possible the purification of pig liver plasma membranes in high yield (1 mg membrane protein per g liver, wet weight) and within 7 hrs. It is assumed that the purity of the membranes prepared in this way is similar to that described by Lesko [12]; the conventional enzyme markers have not been checked nor have the membranes been submitted to electron microscopy, as the only requirement the preparation should meet was specific binding of glucagon.

Sufficient sensitivity of the receptor-assay was obtained by 1) the use of large standard- or sample-volumes, which increased the absolute amount of hormone available for binding, and 2) preincubation of membranes with standards or samples before addition of labelled hormone; because of the almost irreversible binding of the hormone in buffer milieu, this system resembles back-titration techniques [27] more than competitive protein-binding.

The percentage bound in the system is low, only a few per cent. Repeated washes of the membranes (a minimum of three times is required), however, ensure adequate precision of the assay. Furthermore, a high percentage bound can be obtained, as binding is a simple function of the amount of membranes present; 70% binding of the label was found in an experiment with large amounts of membrane. As these experiments were rather prodigal of material, no attempts were made to increase this value further. With excess antiserum 95% of the label was bound. The detection limit of the system (40 pg/ml) is below the concentrations of circulating glucagon usually reported [28].

The equilibrium constant (7×10^9 l/mol) and the binding capacity (0.5 pmol/mg membrane protein) determined from the present experiments differ from the corresponding values obtained with rat liver membranes [29]. The values, however, clearly depend on incubation conditions (and degradation of membranes), and the system hardly reaches true equilibrium. Surprisingly, the equilibrium constant for binding of labelled glucagon alone was larger than that obtained with unlabelled glucagon. The two experiments are not quite comparable, but the results are consistent with the finding of Bromer et al. [30] that iodoglucagon has increased biological activity compared with unsubstituted glucagon.

Binding of Enteroglucagon

The glucagon-like activity of extracts of gastrointestinal mucosa, first described by Sutherland and DeDuve [31], later believed to possess glucagon-like immunoreactivity as well [32], has been named gut glucagon [33], gut Glucagon-Like-Immunoreactivity [34] or enteroglucagon [35]. Neither the chemical nature, nor the metabolic role of enteroglucagon is understood at present. A number of components of different molecular weight and charge, and several biological activities have been proposed [4, 5, 36].

Identity between the glycogenolytic factor of Sutherland [37] and one of the fractions of the immunoreactive substances has been suggested [5], but this has been difficult to prove [6, 8, 37]. Furthermore, the discovery of VIP (Vasoactive Intestinal Polypeptide) [38], which has about 50% of the glycogenolytic potency of glucagon and is present in large amounts in extracts of intestinal mucosa [9, 39], renders this suggestion even more hypothetical. Recently the presence of a glucagon-like substance indistinguishable from pancreatic glucagon by immunochemical and physico-chemical methods, in the fundic mucosa of the canine ventricle has been reported by

Unger [40]. This substance may be the original glyco-genolytic factor which was also extracted from canine ventricle. There have been no reports, however, on the presence of an analogous substance in the porcine or human gastrointestinal tract. The enteroglucagon preparations employed in the present investigation contained less than 3% of pancreatic glucagon-like immunoreactivity, compared to the total immuno-reactivity, when measured with an antiserum relatively specific for pancreatic glucagon (4317) [18].

Bataille et al. [7, 9] have shown that crude extracts interfere with binding of labelled glucagon to rat liver membranes; this was not noted by Pohl [8] or Mur-phy et al. [6], the latter employing a purified prepara-tion. In the present study, three different preparations of enteroglucagon were analysed; all were porcine and analysed with porcine hepatic plasma-mem-branes. While MUC 101 yielded linear dilutions and superimposability to standards in the radioimmuno-assay (thereby permitting quantification), the Kenny-extracts, and hence the purified product thereof, did not. There are two possible explanations of this, both of which may be contributory: 1) enteroglucagon is rapidly degraded to fragments of less immunoreac-tivity at room temperature [21], and 2) the precipita-tion techniques employed in the preparation of MUC-101 may irreversibly destroy immunoreactivity of fragments, or components, that do not give linear dilutions in the radioimmunoassay. The pattern of immunoreactivity obtained with the purified entero-glucagon after polyacrylamide gel-electrophoresis sug-gests some heterogeneity of the preparation consistent with the results of Murphy et al. [6].

However, the aim was to investigate the effect of total enteroglucagon, that is the total immunoreac-tivity of the porcine intestinal mucosa, and constantly a concentration-dependent inhibition of the binding of ^{125}I-labelled glucagon was observed. That this is the case with the purified enteroglucagon also indicates that the inhibition is not merely non-specific inter-ference, nor an effect of other hormones present in the crude extracts. Furthermore, when tested for cross-reaction with other available peptides, the receptors appeared highly specific. The crossreaction noted with 10% pure cholecystokinin is fully explained by the enteroglucagon-content of this preparation. In accordance with the results of Bataille et al. [7, 9], enteroglucagon was found to bind to the membranes with lower affinity than glucagon; depending on the preparation, 2−4 times more enteroglucagon was needed to reduce binding of labelled glucagon by 50%. This is consistent with the data of Bataille et al. [7] for the fraction "B". In spite of the lower affinity, physiological concentrations of enteroglucagon are readily detected in the receptor-assay; concentrations higher than 500 pg equivalents/ml (Fig. 11) are seen after fat-meals [11],during oral glucose tolerance tests [21] and under various pathological conditions [42, 43].

The results indicate that enterglucagon may bind to the hepatic receptors in vivo also; but this does not necessarily mean that the enteroglucagon will stim-ulate adenyl-cyclase or promote glycogenolysis. Firstly, not all receptors seem to be connected with adenyl-cyclase activity [44]; this, however, may be an artefact of preparation [45]. Secondly, glucagon fragments, shown to interfere with binding, may not stimulate adenyl-cyclase [46, 47]. The activation described by Bataille et al. [7, 9] may result from the action of coextracted VIP or of other not yet charac-terized hormones. It has been proposed that increased release of enteroglucagon during reactive hypogly-cemia might competitively inhibit glucagon-stimulated glycogenolysis [4, 43]; this hypothesis gains some support from the present results.

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