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Receptors for Pancreastatin in Rat Liver Membranes: Molecular Identification and Characterization by Covalent Cross-Linking

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SUMMARY

High affinity binding sites for pancreastatin were identified for the first time, and their molecular characterization was performed with rat liver membranes. Using rat ^{125}I-pancreastatin, we have studied the interaction of pancreastatin with liver membranes. Cross-linking of the tracer to the membranes was performed using the bifunctional reagent dithiobis(succinimidyl propionate). Analysis of binding under equilibrium conditions indicated the existence of one class of binding sites, with a B_{max} of 15 fmol/mg of protein and an apparent K_d of 0.2 nM. The cross-linking of ^{125}I-pancreastatin to liver membranes revealed a single band of M_r 40,000, corresponding to the ^{125}I-pancreastatin-receptor complex. The labeling of this complex was inhibited in the presence of rat pancreastatin (10^{-10} to 10^{-7} M) and in the presence of guanyl-5′-ylimidodiphosphate (10^{-7} to 10^{-4} M). Pretreatment of rat liver membranes with pertussis toxin did not affect pancreastatin binding or the inhibition by guanyl-5′-ylimidodiphosphate of pancreastatin binding. The specificity of pancreastatin binding was further assessed by displacement experiments with pancreastatin from other species and vasopressin. The binding of the pancreastatin-receptor complexes to Sepharose coupled to different lectins showed the glycoprotein nature of the pancreastatin receptor. These results strongly suggest that rat liver possesses a specific pancreastatin receptor, a glycoprotein of M_r 35,000 that is coupled to a pertussis toxin-insensitive G protein in the plasma membrane.

Pancreastatin, a peptide containing 49 amino acids with a carboxyl-terminal glycinamide, was isolated from porcine pancreas by Tatemoto *et al.* (1), and its role as a regulatory pancreatic hormone has been established in light of a variety of biological effects that could be assigned to the carboxyl-terminal part of the molecule. These effects are exerted on endocrine and exocrine pancreatic secretion (2–7), gastric secretion (8), parathormone release (9), plasma catecholamine levels (10), and memory retention (11). From several lines of evidence, it is now established that pancreastatin arises from proteolytic cleavage of its precursor chromogranin A, a glycoprotein present in endocrine and neuronal cells (12, 13). Rat chromogranin A cDNA revealed the existence of a pancreastatin-like sequence, homologous to porcine pancreastatin (14–17). Synthetic rat pancreastatin has also been shown to have biological activity in different tissues (10, 18–20).

We previously reported that pancreastatin produces hepatic glycogenolysis both *in vivo* and *in vitro* (21–23), an effect that is dependent on calcium. Recently, we have also studied the effect of rat pancreastatin on intracellular calcium levels in rat hepatocytes (24). This effect of pancreastatin to increase intracellular calcium levels proved to be mediated by both pertussis toxin-sensitive and -insensitive pathways. The purpose of the present study was to examine the molecular mechanism underlying the glycogenolytic effect of pancreastatin on rat liver, by identifying and characterizing the pancreastatin receptor in rat liver membranes.

In this paper, we demonstrate for the first time the presence of pancreastatin receptors and we present their molecular characterization. We have used rat ^{125}I-pancreastatin to identify the ligand binding of the pancreastatin receptor in rat liver, and we have used the homobifunctional cross-linking reagent DSP to further characterize the molecular structure of the receptor. We show that rat liver membranes contain one class of binding sites, with a B_{max} of 15 fmol/mg of protein and an apparent K_d of 0.2 nM. We demonstrate that the high affinity pancreastatin receptor exists as a monomeric glycoprotein with an apparent molecular weight of 35,000 that is functionally coupled to a pertussis toxin-insensitive G protein.

Materials and Methods

Reagents. Rat [Tyr^0]-pancreastatin [[Tyr^0]-chromogranin-A(264–314) amide], rat pancreastatin, porcine pancreastatin(1–49), porcine

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ABBREVIATIONS: DSP, dithiobis(succinimidyl propionate); DSS, disuccinimidyl suberate; DST, disuccinimidyl tartrate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GMP-P(NH)P, guanyl-5′-ylimidophosphate; WGA, wheat germ agglutinin; Con A, concanavalin A; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

pancreastatin(33–49), and human pancreastatin [human chromogranin A(286–301) amide] were provided by Peninsula Laboratories Europe (Merseyside, UK). Arg-vasopressin was from Boehringer Mannheim (Mannheim, Germany). Pertussis toxin, bacitracin, dithiothreitol, bovine serum albumin (fraction V), GTP, GMP-P(NH)P, and other nucleotides were from Sigma Chemical Co. (St. Louis, MO). The cross-linkers DSP, DSS, and DST were from Pierce Chemical Co. (Rockford, IL). Electrophoretic chemicals were from Bio-Rad Laboratories (Richmond, CA). Molecular weight standards were from Pharmacia (Uppsala, Sweden). Carrier-free $Na^{125}I$ (IMS 30, 100 mCi/ml) was from Amersham Radiochemical Center (UK). Tyrosylated rat pancreastatin was radioiodinated with ^{125}I (Amersham), using the chloramine T method (25), to a specific activity of 1500 Ci/mmol and was purified by gel filtration (Sephadex G-50).

Membrane preparation. Animal used were male Wistar rats (150–200 g) fed *ad libitum*. Rat liver membranes were prepared according to the method of Neville (26) up to step 11.

Pertussis toxin treatment of liver membranes. The treatment of membranes with pertussis toxin was carried out as described by Ribeiro-Neto *et al.* (27). Control membranes were treated in an identical manner except that there was no toxin present in the incubation. The membrane suspension was then centrifuged and the pellet was washed twice and finally resuspended in HEPES buffer (20 mM, pH 7.5) containing bacitracin (100 μg/ml) and leupeptin (100 μg/ml).

Binding studies. Rat liver membranes (0.5 mg/ml protein) were incubated with 5×10^{-11} M ^{125}I-pancreastatin in 20 mM HEPES, 0.1% (w/v) bacitracin, 0.1% (w/v) leupeptin, 1% (w/v) bovine serum albumin, pH 7.4, for 90 min at 15°. For saturation analyses, increasing concentrations of native peptide (10^{-11} to 10^{-7} M) were incubated under the aforementioned conditions. For the competitive inhibition study, increasing concentrations of the related peptides were used. At the end of incubation, 200-μl aliquots were mixed with 200 μl of ice-cold incubation buffer and centrifuged at 20,000 × *g* for 5 min. The supernatant containing the free radioactivity was rapidly removed by aspiration, and the pellets were washed twice with 20 mM HEPES buffer, pH 7.4, containing 10% (w/v) sucrose. The radioactivity was counted in a LKB γ counter. Nonspecific binding routinely represented 25–30% of total binding and was substracted from the total binding to calculate the specific binding.

Cross-linking conditions. Membranes (0.5 mg/ml) were incubated with 0.2 nM ^{125}I-pancreastatin and other effectors, as stated in Results, in 5 ml of 20 mM HEPES buffer containing 0.1% (w/v) bacitracin and 0.1% (w/v) leupeptin, pH 7.4, for 90 min at 15°. Membranes were then pelleted by centrifugation at 20,000 × *g* for 10 min and were washed twice in 20 mM HEPES buffer, pH 7.4. Nonspecific binding routinely represented 25–30% of total binding. The final pellet was resuspended in 1 ml of 20 mM HEPES, pH 7.4, and DSP was added at a final concentration of 1 mM, from a 50 mM stock solution in dimethylsulfoxide. The reaction was carried out for 20 min at 4° and stopped by addition of 20 μl of ice-cold 1 M Tris, pH 6.8. The mixture was centrifuged at 4° for 15 min at 20,000 × *g*, and the resulting pellet was suspended by five successive passages through a 25-gauge needle in 60 mM Tris·HCl buffer, pH 8.8, containing 10% (v/v) glycerol, 0.001% (w/v) bromphenol blue, and 3% (w/v) SDS. After heating for 30 min at 80°, the suspension was centrifuged for 15 min at 20,000 × *g* and the supernatant was subjected to SDS-PAGE.

Adsorption to lectins. Lectins coupled to agarose beads (about 2 mg of settled protein-coupled gel; Sigma) were used to investigate the glycoprotein nature of the pancreastatin receptor. Lectins were washed in 20 mM HEPES buffer, pH 7.4, containing 0.1 M NaCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$, and 0.1% Triton X-100. The ^{125}I-pancreastatin-cross-linked membranes were solubilized for 1 hr at 4° and centrifuged at 100,000 × *g* for 1 hr. The Triton X-100-extracted ^{125}I-pancreastatin-receptor complex was then added to 50 μl of lectin-agarose preparation and incubated in the aforementioned buffer for 30 min at 20°, with gentle agitation. Gels were washed two times with 1 ml of the same buffer, and the radioactivity adsorbed to the beads was counted. The nonspecific adsorption (25–30% of total binding to lectin) evaluated in the presence of an excess of sugar (0.3 M) was subtracted from the reported values; methyl α-D-mannopyranoside was used in the case of pea agglutinin and Con A, *N*-acetyl-D-glucosamine in the case of WGA, and *N*-acetyl-D-galactosamine in the case of soybean agglutinin.

SDS-PAGE analysis and autoradiography. SDS-PAGE was performed according to the procedure of Laemmli (28). Samples were analyzed on a 12% polyacrylamide slab gel (1.5-mm thickness) with a 5% polyacrylamide stacking gel. Gels were calibrated with the following proteins of known molecular weight: phosphorylase *b* (M_r 94,000), bovine serum albumin (M_r 67,000), ovalbumin (M_r 43,000), carbonic anhydrase (M_r 30,000), soybean trypsin inhibitor (M_r 20,100), and α-lactalbumin (M_r 14,300). The gels were fixed in 43% (v/v) methanol/1.6 M acetic acid, stained in 0.1% (w/v) Coomasie brilliant blue R-250, and destained in the same methanol acetic acid solution described above. The gels were dried and exposed for 7–15 days, at −80°, to DuPont Cronex-4 film with an intensifying screen (DuPont Cronex Lightning Plus).

Data analysis. Analysis of competition and saturation experiments was performed with the EBDA/LIGAND program. The cross-linking experiments were performed at least three times with two different membrane preparations obtained from 10–12 rats. Representative experiments are shown.

Protein determination. Proteins were measured by the procedure of Bradford (29) with a Bio-Rad kit, using bovine serum albumin as a standard.

Results

Binding properties of pancreastatin with liver membranes. Using ^{125}I-labeled rat pancreastatin, we examined the binding characteristics of pancreastatin in rat liver membranes. The specific high affinity binding of ^{125}I-pancreastatin was dependent on time and temperature and highly dependent on pH, reaching a maximum at 15°, 90 min, and pH 7.4, and showed a marked reduction with changes as small as 0.5 pH unit (data not shown). The concentration dependence of pancreastatin binding at equilibrium was determined by addition of increasing concentrations of unlabeled pancreastatin. As shown in Fig. 1, rat pancreastatin in the concentration range between 10^{-11} and 10^{-8} M competitively inhibited the binding

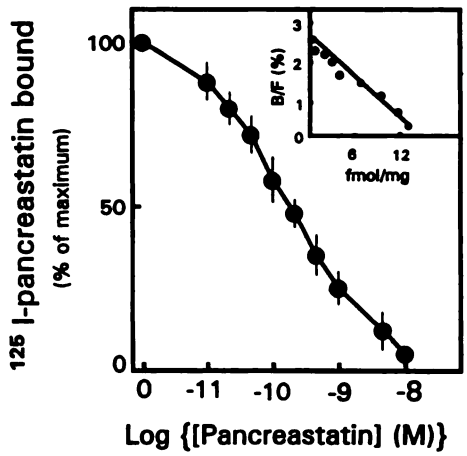

Fig. 1. Inhibition of ^{125}I-pancreastatin binding to liver membranes by unlabeled pancreastatin. Membranes were incubated with ^{125}I-pancreastatin and increasing concentrations of unlabeled pancreastatin under standard conditions, as indicated in Materials and Methods. Specific binding is expressed as the percentage of maximum binding measured in the presence of tracer alone. Each *point* is the mean ± standard error of five separate experiments. *Inset*, Scatchard analysis of the pancreastatin binding data.

of ^{125}I-pancreastatin to liver membranes; half-maximal inhibition was observed at 0.2 nM. Scatchard analysis (30) of the data gave a straight line (Fig. 1, *inset*), indicating the presence of a single site with an apparent K_d of 0.2 ± 0.05 nM; the concentration of binding sites was 15 ± 2 fmol/mg of protein.

The fine specificity of the pancreastatin receptor was studied by using homologous peptides from other species, i.e., pig and human (Fig. 2). Both porcine pancreastatin(33–49) and pancreastatin(1–49) were able to inhibit tracer binding, but with about 30- and 50-fold lower potency (K_d values of 6 and 10 nM, respectively). Human pancreastatin was also able to inhibit the tracer binding, but with about 300-fold lower affinity (K_d of 60 nM). However, neither vasopressin, which shares the last two carboxyl-terminal residues with pancreastatin, nor other unrelated peptides, such as glucagon or vasoactive intestinal peptide, showed any effect at concentrations up to 10^{-6} M (data not shown).

Cross-linking of ^{125}I-pancreastatin to liver membranes and molecular characterization. To further analyze the pancreastatin receptors in rat liver, ^{125}I-pancreastatin was covalently cross-linked to the receptor with three homobifunctional hydroxysuccinimide esters, i.e., DSP, DSS, and DST. After the cross-linked materials were subjected to SDS-PAGE, autoradiography revealed a single band, corresponding to the migration of a protein of M_r 40,000, regardless of the cross-linking agent (Fig. 3). The intensity of the labeling of the M_r 40,000 component was slightly higher with DSP than with DSS or DST. Additional cross-linking experiments were conducted with DSP. When covalently labeled membrane proteins were solubilized under reducing conditions, i.e., in the presence of 100 mM dithiothreitol or β-mercaptoethanol, there was no detectable modification of the electrophoretic mobility of the M_r 40,000 band (Fig. 4). Higher concentrations of dithiothreitol or β-mercaptoethanol (0.4–0.7 M) caused the disappearance of the band by reducing the disulfide bond of DSP (data not shown). Assuming that one molecule of ^{125}I-pancreastatin (M_r 5000) is bound per molecule of receptor, the average molecular weight of the binding protein that is covalently linked to ^{125}I-pancreastatin is 35,000.

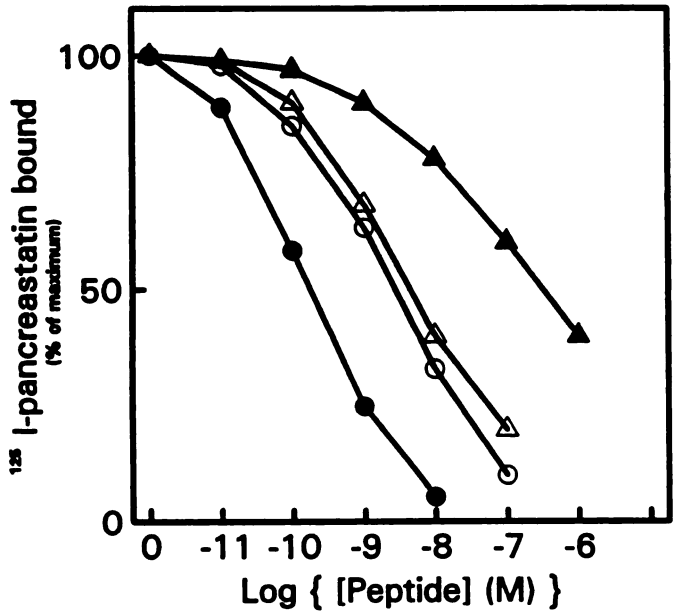

Fig. 2. Competitive inhibition of rat ^{125}I-pancreastatin binding to rat liver membranes by pancreastatin from other species. Conditions were as described in Materials and Methods and in the legend to Fig. 1. Each *point* is the mean of five experiments. Standard errors were always <10%. Dose-response curves for rat pancreastatin (●), porcine pancreastatin(33–49) (○), porcine pancreastatin (△), and human pancreastatin (▲) inhibition of rat ^{125}I-pancreastatin binding to rat liver membranes are shown.

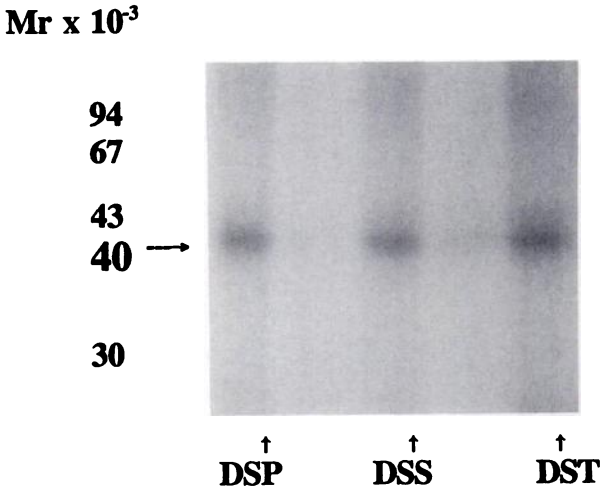

Fig. 3. Effect of various cross-linkers on cross-linking of ^{125}I-pancreastatin to rat liver membranes. Rat liver membranes were incubated with ^{125}I-pancreastatin as specified in Materials and Methods. After separation from free peptide, peptide-bound membranes were treated with various cross-linkers, i.e., DSP, DSS, or DST (final concentration, 1 mM), for 20 min at 4°. Samples were subjected to SDS-PAGE under nonreducing conditions. The autoradiogram of a dried gel is shown.

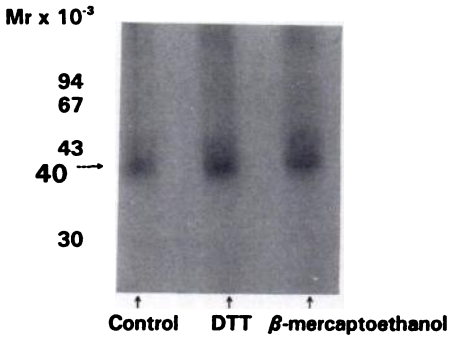

Fig. 4. Effect of reducing conditions on the cross-linking of ^{125}I-pancreastatin to rat liver membranes. Membranes (0.5 mg/ml) were incubated with ^{125}I-pancreastatin as specified in Materials and Methods. After separation from free peptide, peptide-bound membranes were treated with 1 mM DSP and subjected to SDS-PAGE, under nonreducing conditions (control) or after solubilization with 100 mM dithiothreitol (*DTT*) or 100 mM β-mercaptoethanol for 30 min before electrophoresis. The autoradiogram of a dried gel is shown.

Fig. 5 shows that the labeling of the M_r 40,000 component was progressively inhibited by increasing concentrations of unlabeled pancreastatin at concentrations (10^{-10} to 10^{-7} M) that are in the dose range for stimulating glucose production (23), increasing intracellular calcium levels (24), and saturating the pancreastatin receptor, as defined by Scatchard analysis of stoichiometric data (Fig. 1).

To determine the specificity of the M_r 40,000 pancreastatin-binding component, several structurally related peptides were tested for their ability to inhibit ^{125}I-pancreastatin binding to liver membranes receptors. Vasopressin did not interact with the pancreastatin receptor, and pancreastatin from other species partially inhibited the appearance of the M_r 40,000 com-

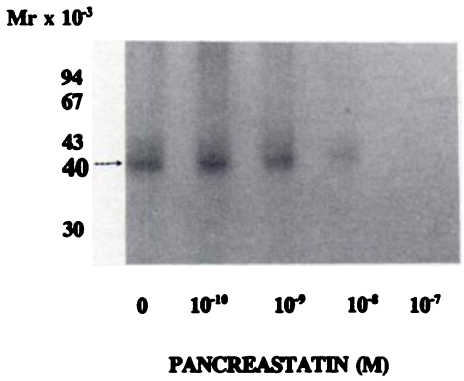

Fig. 5. Effect of increasing concentrations of unlabeled pancreastatin on the labeling of the M_r 40,000 band. Membranes (0.5 mg/ml) were incubated with ^{125}I-pancreastatin and the indicated concentrations of unlabeled pancreastatin. After the binding reaction and treatment with 1 mM DSP, membranes were solubilized under nonreducing conditions and electrophoresed. The autoradiogram of a dried gel is shown.

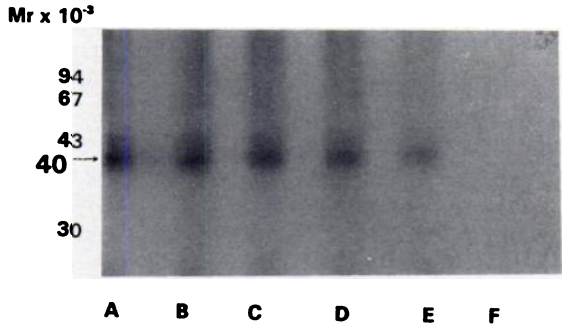

Fig. 6. Specificity of cross-linking of ^{125}I-pancreastatin to rat liver membranes. Membranes were incubated with ^{125}I-pancreastatin in the absence (*lane A*) and in the presence of 10^{-7} M vasopressin (*lane B*), human pancreastatin (*lane C*), porcine pancreastatin(1–49) (*lane D*), porcine pancreastatin(33–49) (*lane E*), and unlabeled rat pancreastatin (*lane F*). The peptide-bound membranes were treated with 1 mM DSP, solubilized under nonreducing conditions, and electrophoresed. The autoradiogram of a dried gel is shown.

ponent (Fig. 6), with the following order of potency: rat pancreastatin ≫ porcine pancreastatin(33–49) > porcine pancreastatin(1–49) > human pancreastatin.

Guanine nucleotides inhibited the binding of pancreastatin to rat liver membranes (data not shown), and the order of potency was GMP-P(NH)P > GTP > GDP. The inhibition of the labeling of the M_r 40,000 component by guanine nucleotides resulted in the same order of potency (data not shown). Moreover, GMP-P(NH)P was found to reduce the labeling of the M_r 40,000 component in a dose-dependent manner between 10^{-7} and 10^{-4} M (Fig. 7). Pertussis toxin pretreatment of liver membranes before cross-linking of ^{125}I-pancreastatin did not alter the intensity or the mobility of the labeled M_r 40,000 band (Fig. 7). In addition, the ability of GMP-P(NH)P to inhibit the labeling of the band was not affected by the previous treatment of membranes with pertussis toxin (data not shown).

Because many hormone receptors are glycoproteins, the 1% Triton X-100 extract of membranes containing receptors covalently attached to ^{125}I-pancreastatin was incubated with various lectins coupled to Sepharose. Radioactivity was specifically adsorbed by several lectins, with the following order of potency (Fig. 8): soybean agglutinin > pea agglutinin > WGA > Con A; the specificity of adsorption was assessed by the elution of the radioactivity from the beads with *N*-acetyl-D-galactosamine in

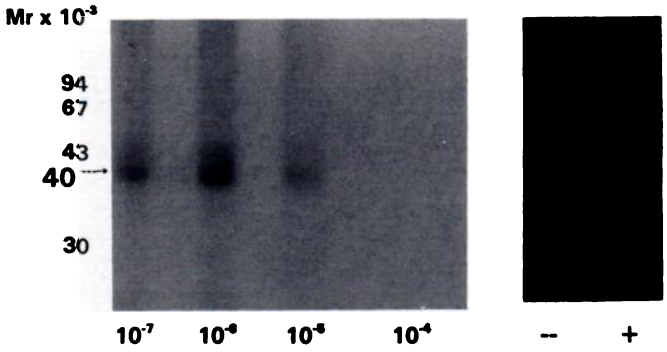

Fig. 7. Effect of increasing concentrations of GMP-P(NH)P on the labeling of the M_r 40,000 band. *Left*, membranes (0.5 mg/ml) were incubated with ^{125}I-pancreastatin and the indicated concentrations of GMP-P(NH)P. After binding reactions and treatment with 1 mM DSP, membranes were solubilized under nonreducing conditions and subjected to electrophoresis. The autoradiogram of a dried gel is shown. *Right*, membranes were preincubated with or without pertussis toxin (2 μg/ml) before binding incubations and cross-linking experiments, as described in Materials and Methods. The autoradiogram of a dried gel is shown.

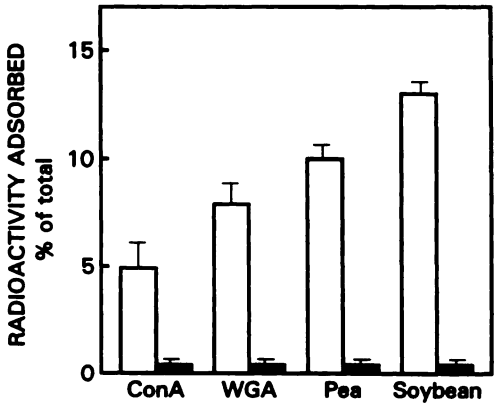

Fig. 8. Adsorption of solubilized ^{125}I-pancreastatin-receptor complex to lectin-Sepharose. Membranes (0.5 mg/ml) were incubated with ^{125}I-pancreastatin. After separation of peptide-bound membranes and treatment with 1 mM DSP, membranes were solubilized with 1% (v/v) Triton X-100. The supernatant was then applied to various lectins coupled to Sepharose, as indicated in Materials and Methods. Data are expressed as radioactivity specifically bound to lectins before (□) and after (■) elution with 0.3 M *N*-acetyl-D-galactosamine in the case of soybean agglutinin, methyl α-D-mannopyranoside in the case of pea agglutinin and Con A, and *N*-acetyl-D-glucosamine in the case of WGA. Results are the mean ± standard error of three different experiments performed in duplicate.

the case of soybean agglutinin, methyl α-D-mannopyranoside in the case of pea agglutinin and Con A, and *N*-acetyl-D-glucosamine in the case of WGA.

Discussion

The biological activity of pancreastatin has been demonstrated in pancreatic and extrapancreatic tissues (see Ref. 31 for review). However, the existence of specific receptors has not been demonstrated thus far. For the first time, we have described the existence of pancreastatin-specific high affinity receptors in a target tissue. We previously showed the glycogenolytic effect of pancreastatin in the rat (21–23), an effect that is independent of cAMP and dependent on calcium (23). Moreover, pancreastatin increases intracellular calcium levels

in rat hepatocytes in a dose-dependent manner within the range of effective concentrations (24). To further characterize the molecular basis of this action, we studied the existence of specific pancreastatin receptors in rat liver membranes by using radiolabeled pancreastatin. The present work also describes the molecular characterization of the pancreastatin receptor in rat liver membranes. Analysis of binding experiments at equilibrium indicates the existence of one class of binding sites, with a capacity of 15 fmol/mg of membrane protein and an apparent K_d of about 0.2 nM. This value correlates with the half-maximal concentration (ED_{50}) for the pancreastatin effect on rat liver cells and is comparable to values for most peptidic hormone receptors (32). It is also compatible with the pancreastatin plasma levels measured after a meal (33), consistent with a physiological role of pancreastatin as a regulatory hormone. Moreover, a role for chromogranin A as a prohormone precursor of pancreastatin and other peptides has been suggested (31, 34). Thus, chromogranin A released from the adrenal medulla may be useful to generate, in the periphery, a peptide with a glycogenolytic effect (21–24), as well as a counterregulatory effect on insulin-stimulated glycogen synthesis (35), which could be important in the physiology of the stress.

This study also documents some molecular properties of the pancreastatin receptor protein. By using the homobifunctional reagent DSP we have cross-linked ^{125}I-pancreastatin to liver membranes, identifying a pancreastatin receptor protein with an apparent molecular weight of 35,000. The mobility of the band was not affected by 100 mM dithiothreitol, suggesting that the pancreastatin receptor does not contain any inter- or intrachain disulfide bonds within its structure. However, it should be pointed pointed out that the size of the cross-linked receptor peptide is relatively small, compared with other G protein-coupled receptors (32), which are usually 60–90 kDa. A possible explanation may be proteolysis of the receptor during the preparation of rat liver plasma membranes, which takes a long time and much manipulation to guarantee the complete integrity of the membrane proteins, even in the presence of protease inhibitors (EDTA, bacitracin, and leupeptin were used). Nevertheless, the binding data clearly demonstrate high affinity binding sites that are inhibited by guanine nucleotides. Therefore, the cross-linked receptor peptide seems to contain both the binding site and the site of interaction with G proteins.

The specificity of labeling of the complex was assayed by measuring the interference of rat pancreastatin (10^{-10} to 10^{-7} M) with incorporation of labeled pancreastatin into the membrane M_r 40,000 complex. We found a good correlation between the affinities of several related peptides and their functional potency in glucose production and calcium mobilization in rat hepatocytes (24), with an order of rat pancreastatin ≫ porcine pancreastatin(33–49) > porcine pancreastatin(1–49) > human pancreastatin (Fig. 3). The differences in the amino acid sequences of the different pancreastatin forms may account for the different affinities observed in the binding studies. The slightly higher affinity of porcine pancreastatin(33–49), compared with the complete peptide, may be explained by the fact that the carboxyl-terminal fragment of porcine pancreastatin has greater homology with the amino acid sequence of rat pancreastatin. Vasopressin, which contains the same carboxyl-terminal Arg-Gly-NH_2 and has the same effect as pancreastatin, failed to alter the binding of pancreastatin or the appearance of the labeled band.

As expected for a peptide receptor, the pancreastatin receptor appears to be a glycoprotein, as assessed by specific lectin adsorption. It is noteworthy that the lectins did not affect the binding of ^{125}I-pancreastatin to rat liver membranes, suggesting that the carbohydrate moieties are not in the pancreastatin binding site. However, additional experiments will be needed to characterize the glycoprotein nature of pancreastatin receptors. We are now undertaking the solubilization of the pancreastatin receptor in a functional state, and one could take advantage of the glycoprotein nature of the pancreastatin receptor for its purification and could thus further elucidate the molecular structure of the pancreastatin receptor.

The labeling of the M_r 35,000 protein was reduced by different guanine nucleotides, and GMP-P(NH)P proved to be the most potent inhibitor of the labeling, showing dose dependence (10^{-7}-10^{-4} M). However, pretreatment of membranes with pertussis toxin failed to modify the labeling of the membrane complex and to uncouple the inhibition caused by GMP-P(NH)P, suggesting that a pertussis toxin-insensitive G protein may be involved in the cellular signaling transduction of pancreastatin in hepatocytes. These data are consistent with previous observations of activation of GTPase activity by pancreastatin in rat liver plasma membranes (36). Moreover, pancreastatin has also been shown to stimulate phospholipase C activity in rat liver plasma membranes, involving a pertussis toxin-insensitive mechanism (36). On the other hand, pancreastatin has been reported to stimulate a pertussis toxin-sensitive guanylate cyclase activity (36). Therefore, the possible role of a pertussis toxin-sensitive mechanism in the signal transduction of the pancreastatin receptor should not be ruled out.

The aforementioned results indicate that the pancreastatin receptor in rat hepatocytes is a glycoprotein consisting of a single polypeptide unit of M_r 35,000. This membrane component displays the properties of apparent affinity and specificity that are characteristic of the pancreastatin receptor mediating effects on rat hepatocytes, and it seems to be coupled to a G protein.

In conclusion, our findings (Refs. 21–24, 35, and 36 and this paper) support the proposal that pancreastatin has an hormonal function in a target tissue, the liver, far from the place where it is synthesized and secreted, i.e., the endocrine pancreas. However, rat pancreastatin has not yet been isolated and sequenced on the peptide level, although rat chromogranin A cDNA has revealed the existence of a pancreastatin-like sequence (14–17) and pancreastatin-like immunoreactivity has been demonstrated in several rat tissues (37). The presence of specific pancreastatin receptors in rat liver plasma membranes further supports the hypothesis of the presence of pancreastatin as a metabolic hormone in rats.

Acknowledgments

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