JCI The Journal of Clinical Investigation

Glucagon receptor of human liver. Studies of its molecular weight and binding properties, and its ability to activate hepatic adenylyl cyclase of non-obese and obese subjects.

J N Livingston, … , S Ewerth, P Arner

J Clin Invest. 1985;75(2):397-403. https://doi.org/10.1172/JCI111713.

Research Article

The glucagon receptor and the adenylyl cyclase system of human liver membranes were studied in six non-obese and six obese subjects who had elevated insulin and plasma glucagon levels. Analysis of specific glucagon binding by the method of Scatchard demonstrated a linear (monocomponent) plot with a dissociation constant of 2-3 nM, and the binding at low hormone concentrations was sensitive to guanosine triphosphate (GTP). The molecular weight of the glucagon receptor was 63,000 D as determined by an affinity labeling procedure and sodium dodecyl sulfate gel electrophoresis. Affinity labeling of this structure was specific for glucagon and inhibited by GTP. Glucagon stimulated the production of cyclic adenosine monophosphate (cAMP) by human membranes with half-maximal activation elicited by 6 nM hormone. The human cyclase system required GTP to facilitate an optimal glucagon response. NaF (10 mM) also activated the cyclase system and produced the same magnitude of response as maximum glucagon activation. A comparison of the liver adenylyl cyclase system of non-obese and obese subjects was made using glucagon (5 nM and 1 microM) and NaF (10 mM). No significant differences in cAMP production were noted between the two groups, regardless of the agent used to activate the enzyme. These findings agree with the glucagon binding studies that showed similar amounts of binding activity in the membranes from the two groups. […]

Glucagon Receptor of Human Liver

Studies of its Molecular Weight and Binding Properties, and its Ability to Activate Hepatic Adenylyl Cyclase of Non-obese and Obese Subjects

James N. Livingston
Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642

Kurt Einarsson, Lars Backman, Staffan Ewerth, and Peter Arner
Departments of Medicine and Surgery, Huddinge Hospital, and Department of Surgery, Danderyd's Hospital, Stockholm, Sweden S-14186

Abstract

The glucagon receptor and the adenylyl cyclase system of human liver membranes were studied in six non-obese and six obese subjects who had elevated insulin and plasma glucagon levels. Analysis of specific glucagon binding by the method of Scatchard demonstrated a linear (monocomponent) plot with a dissociation constant of 2–3 nM, and the binding at low hormone concentrations was sensitive to guanosine triphosphate (GTP). The molecular weight of the glucagon receptor was 63,000 D as determined by an affinity labeling procedure and sodium dodecyl sulfate gel electrophoresis. Affinity labeling of this structure was specific for glucagon and inhibited by GTP.

Glucagon stimulated the production of cyclic adenosine monophosphate (cAMP) by human membranes with half-maximal activation elicited by 6 nM hormone. The human cyclase system required GTP to facilitate an optimal glucagon response. NaF (10 mM) also activated the cyclase system and produced the same magnitude of response as maximum glucagon activation.

A comparison of the liver adenylyl cyclase system of non-obese and obese subjects was made using glucagon (5 nM and 1 μM) and NaF (10 mM). No significant differences in cAMP production were noted between the two groups, regardless of the agent used to activate the enzyme. These findings agree with the glucagon binding studies that showed similar amounts of binding activity in the membranes from the two groups. Also, there was no influence of either age or sex of the subjects on the adenylyl cyclase response.

In conclusion, human liver membranes contain a glucagon receptor and an adenylyl cyclase system that correspond closely to the well-studied system in animal liver. This system in human obesity is not altered by the ~twofold elevation in plasma glucagon that occurs in this metabolic disorder.

Introduction

Glucagon is an important contributor to the regulation of hepatic fuel production (1). Although the liver is a primary site of glucagon action (2), and several studies in man have demonstrated significant hepatic extraction of the hormone (3–5), very little direct information is available regarding the interaction of glucagon with the human glucagon receptor, the structure of this receptor, or the characteristics for the hormone activation of the adenylyl cyclase system. Two reports have shown the ability of glucagon to stimulate cyclic adenosine triphosphate (cAMP)[1] production by human liver membranes (6, 7), but studies of the glucagon receptor were not described.

Understanding these basic features of glucagon action is important, particularly since in certain diseases like diabetes, glucagon has been implicated in contributing to the overall pathophysiology (8). Obesity is another abnormality in which recent findings suggest an oversecretion of glucagon (9). It is possible that the oversecretion produces changes in glucagon receptor number and/or its ability to activate the adenylyl cyclase system. For example, in animal studies and in vitro experiments, elevations in glucagon reduce the number of glucagon receptors and decrease glucagon action (10, 12). Unfortunately, studies of glucagon action in human obesity have given ambiguous results, as have the studies of animal models for obesity (13).

In the present study, we examined the interaction of glucagon with the glucagon receptor of human liver membranes, the molecular weight of the glucagon receptor, and the ability of the hormone to stimulate adenylyl cyclase. In addition, studies of the effect of this hormone on adenylyl cyclase activity were conducted in liver membranes from obese subjects, to directly evaluate in this disorder an early step in glucagon action.

Methods

Materials. Carrier-free ^{125}I-Na, [^{3}H]cAMP, and [α-^{32}P]ATP were purchased from Amersham Corp., Arlington Heights, IL. Guanosine triphosphate (GTP), ATP, glucagon, creatine phosphate, creatine phosphokinase (type I), myokinase (grade III), Dowex-50 (hydrogen form), and alumina (chromatographic, neutral) were supplied by Sigma Chemical Co., St. Louis, MO.

Human subjects. Six non-obese and six obese subjects were studied (see Table I for clinical data). The non-obese subjects were admitted to the Surgical Department (Karolinska Institute, Stockholm, Sweden) for elective cholecystectomy; otherwise, they were healthy. The obese subjects were admitted for gastric operations for obesity. The body weight of all the subjects was constant during the 3-mo period before admittance, and none was receiving any drugs that affect liver or carbohydrate metabolism. The subjects were maintained as inpatients on a weight-maintaining diet (45% carbohydrate, 20% protein, and

Address correspondence to Dr. Livingston, Dept. of Medicine, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642.

Received for publication 23 July 1984 and in revised form 19 September 1984.

J. Clin. Invest.

0021-9738/85/02/397/07 $1.00
Volume 75, February 1985, 397–403

1. *Abbreviations used in this paper:* cAMP, cyclic adenosine monophosphate; GTP, guanosine triphosphate; M_r, relative molecular weight.

Table I. Clinical Characteristics of the Subjects

Patient number	Sex	Age	Height	Weight	Fasting level			Liver biopsy
					Glucagon	Insulin	Glucose	
			cm	*kg*	*pg/ml*	*μU/ml*	*mM*	
1	M	47	181	78	56.1	6.8	4.5	Normal
2	F	29	164	59	63.9	9.6	5.0	Normal
3	F	49	168	60	62.2	5.4	4.0	Steatose
4	M	70	179	79	53.9	10.8	5.2	Normal
5	F	30	162	61	48.5	10.9	4.8	Normal
6	F	45	163	59	56.9	8.6	4.7	Steatose
Mean		45	170	66	56.9	8.7	4.7	
SE		6	3	4	2.3	0.9	0.2	
7	F	30	161	145	77.8	20.8	4.4	Steatose
8	M	21	190	165	114.5	21.3	6.2	Steatose
9	F	45	162	143	120.4	14.4	3.9	Steatose
10	F	49	161	117	55.5	29.7	4.4	Normal
11	F	23	164	117	107.5	11.3	4.2	Normal
12	F	33	163	108	76.7	18.4	5.0	Normal
Mean		34	167	133	92.1	19.3	4.7	
SE		5	5	9	10.5	2.6	0.3	
*P**		NS	NS	<0.001	<0.01	<0.01	NS	

* Comparison between obese and non-obese subjects.

35% fat) for 3 d before surgery. After an overnight fast, general anesthesia was induced with a short-acting barbiturate and maintained by phenanyl and a nitrous oxide-oxygen mixture. During surgery, a liver biopsy (1 g) was taken from the left lobe of the liver. The biopsy was immediately placed in ice-cold buffer and taken to the laboratory for the isolation of liver membranes.

This study was approved by the Ethical Committee of the Karolinska Institute. Informed consents were obtained from the subjects after they were given a detailed description of the study.

Preparation of liver membranes. The liver biopsy was homogenized in 50 ml of a 1-mM $NaHCO_3$ buffer, pH 7.5, that contained 0.5 mM $CaCl_2$ (14). The homogenate was subjected to centrifugation at 10,000 *g* for 20 min, and the membranes were washed in 0.25 M sucrose, 10 mM Tris buffer, pH 7.4. The membranes were resuspended in this buffer, frozen at −80°C, and shipped to Rochester, NY for further analysis. Protein content in the membrane preparation was determined as previously described (15).

Rat liver plasma membranes were prepared by the two-phase polymer method of Lesko et al. (14).

Glucagon binding studies. Glucagon was radiolabeled with ^{125}I-Na to a specific activity of 0.8–1.0 Ci/μmol by the chloramine-T method (16). Liver membranes (10–20 μg protein) were incubated for 30 min at 21°C in 200 μl of a 20-mM Tris buffer, pH 7.4, that contained 1 mM EDTA, 0.1 g% (wt/vol) bovine serum albumin, and 1 mg/ml bacitracin. ^{125}I-glucagon (0.33–33 nM) was added in the presence or absence of 1 μM native gucagon used to estimate nonspecific glucagon binding. The incubations were terminated and the membranes isolated by the centrifugation method of Rodbell et al. (17). Degradation of ^{125}I-glucagon was estimated by a trichloroacetic acid precipitation method (18).

Adenylyl cyclase assay. The conditions of the adenylyl cyclase assay were essentially those used by Rojas et al. (19). In brief, liver membranes (10–25 μg protein) were incubated for 10 min at 32°C in 50 μl of the following buffer system: 0.1 mM [α-^{32}P]ATP (2,000 cpm/pmol), 5 mM $MgCl_2$, 1 μM GTP, 1 mM EDTA, 0.1% bovine serum albumin, 1 mM [^{3}H]cAMP (10,000 cpm), 20 mM creatine phosphate, 0.2 mg/ml creatine phosphokinase, 0.02 mg/ml myokinase, and 25 mM Tris-HCl, pH 7.5. Termination of the reaction and quantitation of the cAMP produced from ATP were carried out as described in method C of Salomon et al. (20).

Photoaffinity labeling and sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis of the glucagon receptor. Liver membranes (1.0 mg protein) were incubated with ^{125}I-glucagon and other agents, as indicated in Results, in 1 ml of the buffer used in studies of glucagon binding. After a 20-min incubation at 21°C, the membranes were rapidly chilled to 4°C and pelleted by a 3-min centrifugation step in a microfuge (Beckman Instruments, Inc., Fullerton, CA). The membrane pellet was washed twice by resuspension in 1 ml of an ice-cold 20-mM sodium phosphate buffer, pH 7.4, followed by microfuge centrifugation. After the second wash, the pellet was resuspended in the dark in 1 ml of the phosphate buffer that contained 20 μl of a solution of 6.5 mg of *N*-hydroxysuccimidyl-4-azidobenzoate in 1 ml of dimethyl sulfoxide (21). The membranes were incubated in the dark at 4°C for 10 min. The reaction was terminated by the addition of 20 μl of a 2-M Tris-HCl solution, pH 7.4. The membrane suspension was placed in a small petri dish (35-mm diam) on ice. The dish was positioned 15 cm under a sunlamp (275 W and 110–125 V; General Electric Co.) and exposed to light for 10 min with constant shaking. The membranes were pelleted by microfuge centrifugation and dissolved in 2% SDS, 40 mM Tris-HCl buffer, pH 6.8, that contained 5% 2-mercaptoethanol. After being heated at 100°C for 5 min, the samples were subjected to polyacrylamide gel electrophoresis using a 5–12% gradient resolving gel as described by Laemmli (22). After electrophoresis, the gels were fixed in 10% trichloroacetic acid, stained with Coomassie Blue R-250, destained, and dried. The gels were exposed to preflashed film (X-Omatic; Eastman Kodak Co., Rochester, NY) using intensifying screens (X-Omatic; Eastman Kodak Co.). High molecular weight standards (relative molecular weight [M_r] range, 200,000–45,000; Bio-Rad Laboratories, Richmond, CA) were used to estimate the molecular weight of the glucagon receptor.

In vivo determinations. Venous blood for measurements of plasma glucose, insulin, and pancreatic glucagon was obtained after an overnight

fast. Plasma insulin levels were measured using a commercial radioimmunoassay (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, NJ). Pancreatic glucagon was determined in plasma prepared with Trasylol (400 IU/ml) by the method of Heiding (23) using antiserum (E7) supplied by Von Schenck (24). The analyses of glucagon levels were performed on the same occasion for all the subjects.

Statistical methods. The values given are mean±SE. Student's unpaired *t* test was used for statistical comparison.

Results

Human subjects. Table I lists the characteristics of the two groups of human subjects studied. The weight of the subjects in the obese group was ~100% over that of the non-obese group. The fasting insulin and glucagon levels in the obese subjects were approximately double those found in the normal group. One obese subject had a slightly enhanced fasting blood glucose level at the time of the study. However, on two other occasions immediately before the study, his blood glucose concentration was normal. All of the remaining subjects had normal fasting blood glucose levels at the time of the study.

Glucagon binding and the activation of adenylyl cyclase activity. Initial studies indicated that steady-state binding of glucagon (0.33 nM) by human liver membranes was reached by 30 min at 21°C (data not shown). Glucagon degradation under these conditions was ~2%. In these studies, specific glucagon binding was 50% of the total amount of binding. In contrast, specific binding by rat liver membranes was 90% of the total amount bound.

Fig. 1 shows a Scatchard plot of glucagon binding to liver membranes from a non-obese subject. As illustrated, the plot is linear for human membranes and is quite similar to the linear plot found with glucagon binding to rat liver membranes (Fig. 1, insert). The membranes from both species have disassociation constant values for glucagon binding of 2–3 nM. The major difference lies in the amount of glucagon bound per milligram of protein, with the human preparation having much less total glucagon binding activity than the rat membranes. This difference may be due in part to the use of a highly purified preparation of rat liver plasma membranes, whereas the human preparation is not as enriched in this fraction. It is also likely that the difference in the ratio of specific vs. total glucagon binding in the two membrane preparations arises from the difference in purity.

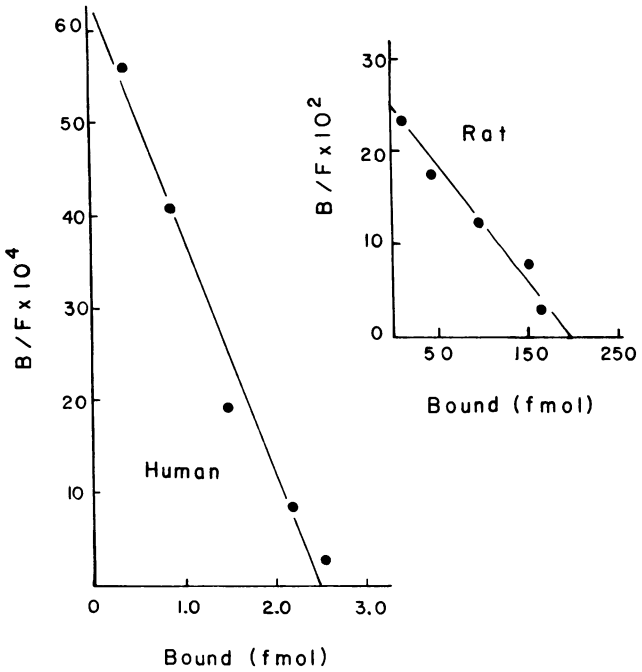

Figure 1. Scatchard analysis of glucagon binding to human and rat liver (insert) membranes. Glucagon binding studies were conducted as outlined in Methods. The Ro values per milligram protein for human and rat membranes were 0.2 and 3.6 pmol, respectively. The disassociation constant values were 2.5 nM and 3 nM for human and rat membranes, respectively.

It was not possible to carry out complete glucagon binding studies on all of the human membrane preparations because of the limited amount of material. Instead, the ability of the membrane fractions from six non-obese and five obese subjects to specifically bind 3 nM ^{125}I-glucagon was compared. The mean for glucagon binding by the non-obese preparations was 1.9±0.3 fmol/20 μg of protein versus a value of 1.5±0.3 fmol for the membranes from the obese subjects. The difference in these values was not statistically significant.

Initial studies of cAMP production in the absence or presence of glucagon demonstrated a linear relationship with respect to the time of incubation (0–20 min) and to the concentration of membranes in the assay (3–24 μg protein) (data not shown). In these studies we also examined the effect of theophylline (3 mM) and found that it did not alter the rate of cAMP production (data not shown). Thus, phosphodiesterase did not destroy a fraction of the cAMP formed during these assays.

The effect of GTP formation on cAMP was studied (Table II), and as indicated, this nucleotide is required to obtain the full stimulation of the cyclase system in the human membranes. In the absence of GTP, glucagon elicited only a 50% stimulation compared with the stimulation found in the presence of the nucleotide. Basal cAMP formation was also affected, being reduced to the lower limit of our ability to accurately measure cAMP formation. Furthermore, GTP at a concentration of 1 μM was sufficient to allow glucagon to maximally activate the cyclase system. Higher nucleotide concentrations did not significantly change basal or glucagon-stimulated cAMP production from the rates found with 1 μM GTP.

GTP also affected glucagon binding by human liver membranes, as reported for membranes from rat liver (19). The specific binding by human membranes of ^{125}I-glucagon (0.33 nM) was reduced to 48±8% of the control value (100%) by 100 μM GTP. At a high hormone concentration of 33 nM, glucagon binding was not altered by the nucleotide (data not shown). Thus, GTP apparently reduces the binding affinity of

*Table II. Effects of GTP on Adenylyl Cyclase Activity**

GTP	− Glucagon	+ Glucagon
μM		
0	0.08	0.60
1	0.21	1.33
10	0.33	1.36
100	0.26	1.35

* pmol/10 μg protein per 10 min. Glucagon concentration was 1 μM.

the glucagon receptor, and enhances glucagon-stimulated activation of human adenylyl cyclase activity.

A comparison of the glucagon dose-response relationship between human and rat liver membranes is shown in Fig. 2. Maximum activation of the adenylyl cyclase system is produced by 0.33 μM glucagon in both membrane preparations. Half-maximal activation in human membranes was produced by a glucagon concentration of ~6 nM, compared with a concentration of 8 nM for rat liver membranes. Note that the amount of protein and the incubation period used in these experiments differed for the two membrane preparations. When standardized to the same membrane concentration and incubation period, basal cAMP formation by human membranes is approximately one-half that found in the rat preparation, and maximal glucagon-stimulated activity is one-third. Again, these quantitative differences between the two membrane preparations are likely due in part to different degrees of enrichment in plasma membranes. As subsequently demonstrated (see Fig. 3), the response of human membranes to 10 mM NaF is quite similar to that produced by maximal glucagon stimulation. This relationship is well established in highly enriched preparations of rat liver plasma membranes (25), which suggests that the lack of purity of the human preparations does not alter their ability to respond to the hormone.

Fig. 3 compares the ability of membranes from non-obese and obese subjects to respond to glucagon and NaF. Two glucagon concentrations were used; one that produces approximately half-maximal stimulation, and another that maximally activates the cyclase system. NaF was included in the study to provide an independent estimate of cyclase content and a separate parameter other than membrane protein to standardize glucagon stimulation. The results indicate similar basal adenylyl cyclase activity between both groups, with no significant differences in cAMP formation with either concentration of glucagon or with NaF stimulation. Thus, the membranes isolated from the obese subjects have a normal response to glucagon, a finding that agrees with normal ^{125}I-glucagon binding.

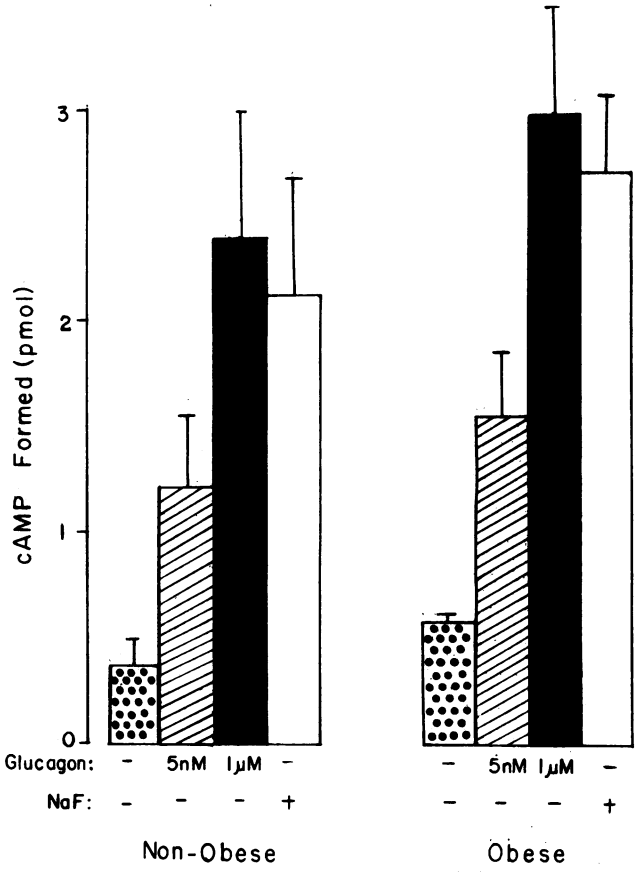

Figure 3. Formation of cAMP by liver membranes from non-obese and obese human subjects. The adenylyl cyclase assay contained 10 μg of membrane protein and the indicated concentration of glucagon or NaF. The incubation was terminated at 10 min. Six obese and six non-obese subjects were studied and the results were expressed as the mean±SE.

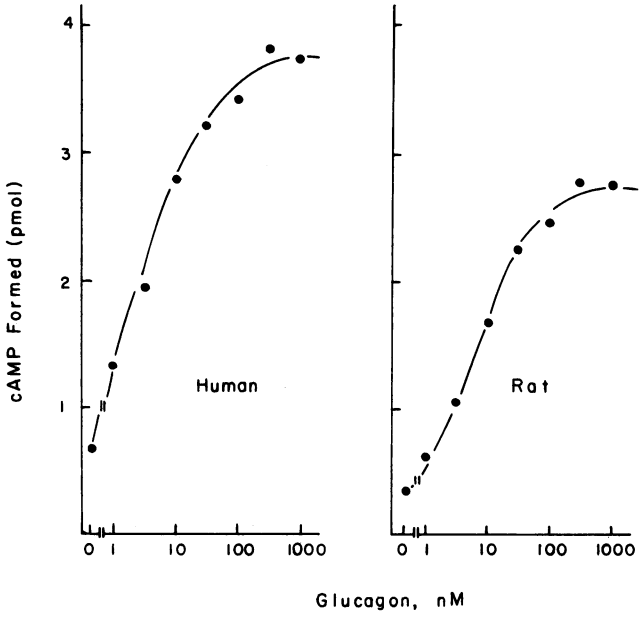

Figure 2. Glucagon dose-response relationship for cAMP formation by human and rat liver membranes. The indicated concentrations of glucagon were incubated with human (12 μg protein) or rat (6 μg) liver membranes in the adenylyl cyclase assay. The incubation was terminated at 10 min for human membranes and at 5 min for the rat liver preparation.

Studies of the structure of the human glucagon receptor. Because of the large amount of membranes required by the structural studies, membranes from four normal subjects were pooled for these studies. Fig. 4 shows an autoradiogram of a polyacrylamide gel after the affinity labeling procedure described in Methods was carried out in human and rat liver membranes. Both membranes contain several structures that are labeled with ^{125}I-glucagon by the photoaffinity labeling technique. However, only one structure in each membrane preparation is specifically labeled with ^{125}I-glucagon, i.e., one whose labeling is markedly reduced by the presence of 1 μM native glucagon. The molecular weight of this structure in rat liver membranes is ~58,000, whereas in human membranes, the affinity labeled component is slightly larger, having a molecular weight of 63,000.

To further characterize the specificity of this binding component for glucagon and to compare it with the established features of the glucagon receptor, studies using native insulin, GTP, and different concentrations of native glucagon were conducted (Fig. 5). As shown, when the membranes were

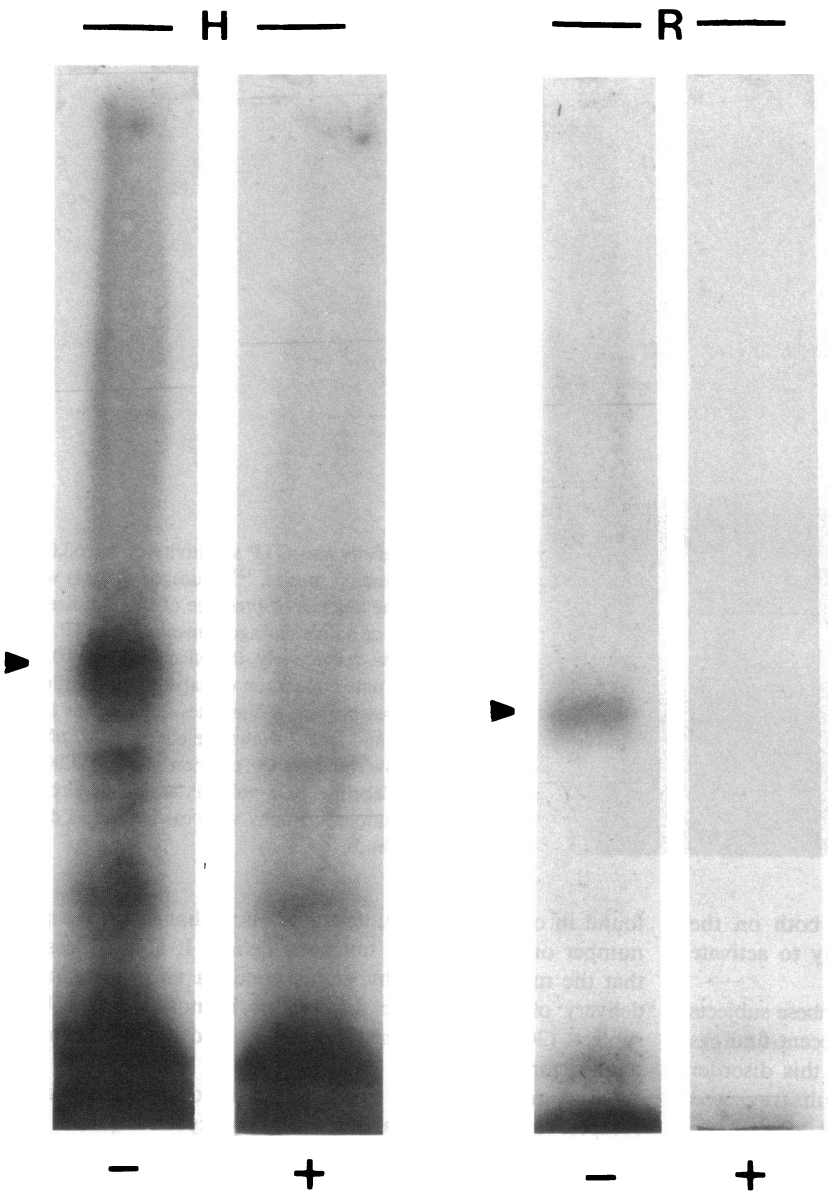

Figure 4. Structural studies of the human and rat liver glucagon receptor. ^{125}I-glucagon (5 nM) was covalently attached to human (H) and rat liver (R) membranes and subjected to SDS polyacrylamide gel electrophoresis (see Methods). The studies were conducted in the absence (−) and presence (+) of 1 μM native glucagon. The arrows indicate specific glucagon labeling. The figure is an autoradiogram of the SDS gel prepared as described in Methods.

incubated in the presence of increasing concentrations of native glucagon, there was a progressive decrease in the amount of the affinity labeled 63,000 band. Laser density spectrophotometric scanning of the autoradiogram demonstrated a 50% reduction in ^{125}I-glucagon labeling of the 63,000 M_r band by 5 nM native glucagon. A high concentration of native insulin, however, did not reduce the glucagon labeling of this structure, in contrast to the incubation with GTP, which caused a marked decrease. These results are compatible with those expected of a glucagon receptor, and provide a strong argument that by polyacrylamide gel electrophoresis, the human glucagon receptor is a structure of 63,000 M_r.

Discussion

These findings demonstrate a glucagon receptor and a responsive adenylyl cyclase system in human liver membranes that are quite similar to the well-characterized system in rat liver membranes (17, 19, 25). Similarities are apparent in the glucagon binding characteristics between human and rat receptors, and both structures also have similar molecular weights. The M_r value of 63,000 for the human receptor is only slightly larger than the M_r 58,000 found in the rat liver membranes. These values are much larger than the 23,000 M_r originally reported by Bregman and Levy (26); however, they agree in general with the more recent reports of the size of the rat glucagon receptor, which range from 52,000 to 70,000 M_r (21, 27, 28).

The adenylyl cyclase system of human membranes also had a similar sensitivity to glucagon stimulation as rat liver membranes. The median effective dose value for stimulation by glucagon was 6 nM, and GTP was required for glucagon to fully activate the cyclase system. This GTP effect differs from the results described by Pecker et al. (7), who stated that the nucleotide did not affect the ability of glucagon to stimulate cAMP production. The reason for this difference is not clear,

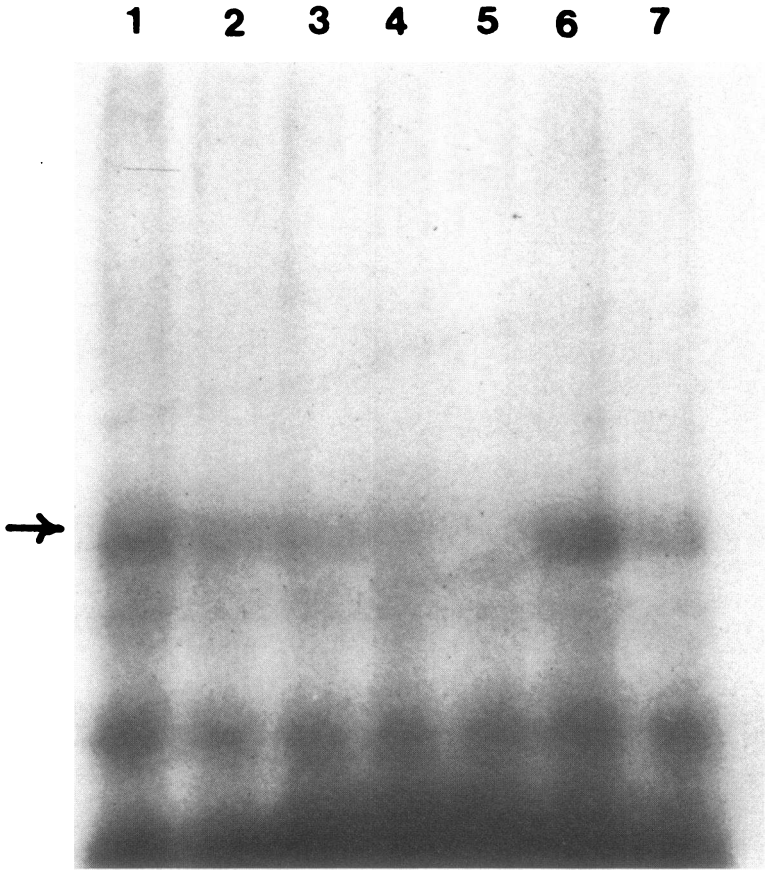

Figure 5. Specificity and GTP sensitivity of the 63,000 M_r glucagon binding species. ^{125}I-glucagon (1 nM) was incubated in the absence or presence of the indicated concentrations of native glucagon, insulin, or GTP. Lanes 1–5 show, respectively, the effects of 0, 5, 10, 100, and 1,000 nM native glucagon on labeling of the glucagon binding protein; lane 6 illustrates the effect of 1 μM native insulin, and lane 7 shows the effect of 100 μM GTP. The arrow indicates the position of the 63,000 M_r band. Affinity labeling, SDS polyacrylamide gel electrophoresis, and autoradiography were carried out as described in Methods.

since our data clearly show an effect of GTP both on the glucagon receptor binding activity and its ability to activate the adenylyl cyclase system.

The ability of human liver membranes from obese subjects to respond to glucagon was examined because recent findings indicate the presence of hyperglucagonemia in this disorder (9). Although other work has given conflicting results (reviewed in 13), the careful studies by Starke et al. (9) clearly demonstrate elevated pancreatic glucagon in obese subjects, including those who were glucose tolerant or intolerant. Our obese subjects also showed evidence of increased secretion. We measured plasma glucagon with a sensitive and specific radioimmunological method (23, 24) and observed almost a 100% increase in the fasting glucagon level in obese subjects compared with non-obese subjects.

The presence of hyperglucagonemia in obesity raises the possibility that alterations exist in the number of glucagon receptors and glucagon action in much the same manner that hyperinsulinemia is associated with insulin resistance and loss of insulin receptors (29). Our findings show, however, that the membranes from obese subjects are as glucagon-responsive as those from normal weight subjects, regardless of the basis for expressing the glucagon effect, i.e., per milligram of protein, or the ability of NaF to stimulate cAMP production. These findings, along with the similar amount of glucagon binding activity in the two groups, indicate an adequate population of glucagon receptors that are appropriately coupled to the adenylyl cyclase system in obesity. Thus, unlike in vitro studies of hepatocytes, in which high concentrations of glucagon cause "down-regulation" of the glucagon receptor and a loss of response to the hormone (10), the moderate hyperglucagonemia found in obesity is not sufficient to elicit changes in receptor number or alterations in hormone action. It is also possible that the mild degree of hyperglucagonemia and the pulsatile delivery of the hormone that occurs in normal and obese subjects (30) helps prevent receptor loss and the development of glucagon resistance.

In summary, human liver membranes contain a glucagon receptor of M_r 63,000 and an adenylyl cyclase system that corresponds closely to the well-studied system in rat liver membranes. Furthermore, the hyperglucagonemia that develops in obesity does not alter the ability of this system to respond to glucagon. These findings do not eliminate the possibility of alterations at sites beyond the cyclase system, e.g., elevated phosphodiesterase activity (31), but they show that in obesity the initial steps in hepatic glucagon action are intact.

Acknowledgments

We thank Dr. G. Lundgrist and Dr. L. Wide (University of Upsala, Sweden) for measuring plasma glucagon levels. This work was supported by National Institutes of Health grant AM25116 and grants from the Swedish Medical Research Council, the Swedish Diabetes Association, Nordic Insulin Foundation, Folksam Foundation, and Osterman Foundation.

References

1. Unger, R. H., and L. Orci. 1981. Glucagon and the A Cell. Physiology and Pathophysiology (Part I). *N. Engl. J. Med.* 304:1518–1524.

2. Sperling, M. A. 1979. Glucagon: secretion and actions. *Adv. Exp. Med. Biol.* 124:29–61.

3. Fischer, M., R. S. Sherwin, R. Hendler, and P. Felig. 1976. Kinetics of glucagon in man: effects of starvation. *Proc. Natl. Acad. Sci. USA.* 73:1735–1739.

4. Walter, R. M., E. N. Gold, C. A. Michas, and J. W. Ensinck. 1980. Portal and peripheral vein concentrations of insulin and glucagon after arginine infusion in morbidly obese subjects. *Metab. Clin. Exp.* 29:1037–1040.

5. Jaspan, J. B., J. Ruddick, and E. Rayfield. 1984. Transhepatic glucagon gradients in man: evidence for glucagon extraction by human liver. *J. Clin. Endocrinol. Metab.* 58:287–292.

6. Israelsson, B., A. Berglund, U. Ljunqsvisr, and J. Malmquist. 1978. Adenylate cyclase activity in human liver membranes and its inhibition by adenosine and adenine nucleotides. *Scand. J. Clin. Lab. Invest.* 38:289–293.

7. Pecker, F., P. Duvaldestin, P. Berthelot, and J. Hanoune. 1979. The adenylate cyclase system in human liver: characterization, subcellular distribution and hormonal sensitivity in normal or cirrhotic adult and in foetal liver. *Clin. Sci. (Lond.).* 57:313–325.

8. Unger, R. H., and L. Orci. 1981. Glucagon and the A cell: physiology and Pathophysiology (Part II). *N. Engl. J. Med.* 304:1575–1580.

9. Starke, A. A. R., G. Erhardt, M. Berger, and H. Zimmermann. 1984. Elevated pancreatic glucagon in obesity. *Diabetes.* 33:277–280.

10. Santos, A., and E. Blazquez. 1982. Direct evidence of a glucagon-dependent regulation of the concentration of glucagon receptors in the liver. *Eur. J. Biochem.* 121:671–677.

11. Srikant, C. B., D. Freeman, K. McCorkle, and R. Unger. 1977. Binding and biologic activity of glucagon in liver cell membranes of chronically hyperglucagonemic rats. *J. Biol. Chem.* 252:7434–7436.

12. Bhathena, S. J., N. R. Voyles, S. Smith, and L. Recant. 1978. Decreased glucagon receptors in diabetic rat hepatocytes. *J. Clin. Invest.* 61:1488–1497.

13. Holst, J. J. 1983. Glucagon in obesity. *In* Handbook of Experimental Pharmacology. Vol. 66. P. Lefebvre, editor. Springer-Verlag New York, Inc., New York. 507–521.

14. Lesko, L., M. Donlon, G. V. Marinetti, and J. D. Hare. 1973. A rapid method for the isolation of rat liver plasma membranes using aqueous two-phase polymer system. *Biochim. Biophys. Acta.* 331:173–179.

15. Simpson, I. A., and O. Sonne. 1982. A simple, rapid, and sensitive method for measuring protein concentration in subcellular fractions prepared by sucrose density ultracentrifugation. *Anal. Biochem.* 119:424–427.

16. Livingston, J. N., P. Cuatrecasas, and D. H. Lockwood. 1974. Studies of glucagon resistance in large rat adipocytes: ^{125}I-labeled glucagon binding and lipolytic capacity. *J. Lipid Res.* 15:26–32.

17. Rodbell, M., M. J. Krans, S. L. Pohl, and L. Birnbaumer. 1971. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. *J. Biol. Chem.* 246:1861–1871.

18. Duckworth, W. C., and A. E. Kitabchi. 1974. Insulin and glucagon degradation by the same enzyme. *Diabetes.* 23:536–543.

19. Rojas, F. J., T. L. Swartz, R. Iyengar, A. J. Garber, and L. Birnbaumer. 1983. Monoiodoglucagon: synthesis, purification by high pressure liquid chromatography, and characteristics as a receptor probe. *Endocrinology.* 113:711–719.

20. Salomon, Y., C. Landos, and M. Rodbell. 1974. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* 58:541–548.

21. Johnson, G. L., V. I. MacAndrew and P. F. Pilch. 1981. Identification of the glucagon receptor in rat liver membranes by photoaffinity crosslinking. *Proc. Natl. Acad. Sci. USA.* 78:875–878.

22. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (Lond.).* 227:680–685.

23. Heiding, L. G. 1971. Radioimmunological determination of pancreatic and gut glucagon in plasma. *Diabetologia.* 7:10–19.

24. Von Schenck, H. 1977. Production and characterization of an antiserum against pancreatic glucagon. *Clin. Chim. Acta.* 80:455–463.

25. Rodbell, M., L. Birnbaumer, S. Pohl, and H. M. Krans. 1971. The glucagon-sensitive adenyl cyclase system in plasma membranes of rat liver. *J. Biol. Chem.* 246:1877–1882.

26. Bregman, M. D., and D. Levy. 1977. Labeling of glucagon binding components in hepatocyte plasma membranes. *Biochem. Biophys. Res. Commun.* 78:584–590.

27. Iyengar, R., and J. T. Herberg. 1984. Structural analysis of the hepatic glucagon receptor: identification of a guanine nucleotide-sensitive hormone-binding region. *J. Biol. Chem.* 259:5222–5229.

28. Demoliou-Mason, C., and R. M. Epand. 1982. Identification of the glucagon receptor by covalent labeling with a radiolabelled photoreactive glucagon analogue. *Biochemistry.* 21:1996–2004.

29. Arner, P., K. Einarsson, L. Backman, K. Nilsell, K. M. Lerea, and J. N. Livingston. 1983. Studies of liver insulin receptors in non-obese and obese human subjects. *J. Clin. Invest.* 72:1729–1736.

30. Hansen, B. C., K-L. C. Jen, S. B. Pek, and R. A. Wolfe. 1982. Rapid oscillations in plasma insulin, glucagon, and glucose in obese and normal weight humans. *J. Clin. Endocrinol. Metab.* 54:785–792.

31. De Santis, R. A., T. Gorenstein, J. N. Livingston, and D. H. Lockwood. 1974. The role of phosphodiesterase in glucagon resistance of large adipocytes. *J. Lipid Res.* 15:33–38.