Hepatic Extraction of Plasma Immunoreactive Glucagon Components

Predilection for 3500-dalton Glucagon Metabolism by the Liver

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SUMMARY

This study examines quantitatively the extraction of plasma immunoreactive glucagon (IRG) components by the liver. It was shown that the liver has a predilection for removal of the 3500-dalton biologically active IRG component with virtually no extraction of the other IRG fractions. Hepatic extraction of whole plasma IRG was 25.2 ± 2.5%. Analysis of the hepatic extraction of the four plasma immunoreactive components, separated by gel filtration, revealed variable but quantitatively insignificant extraction of all components other than the 3500-dalton fraction, which was 33.4 ± 3.2% ($P < 0.001$). Hepatic extraction of whole plasma IRG was significantly less than that of the 3500-dalton component during periods of basal glucagon secretion when IRG fractions other than the 3500-dalton fraction contribute substantially to the whole plasma IRG level. However, during periods of stimulation of glucagon secretion by arginine or arginine plus cholecystokinin-pancreozymin, when the 3500-dalton component accounts for virtually all of the whole plasma IRG level, hepatic extraction of whole plasma IRG was similar to that of the 3500-dalton fraction. DIABETES *30*:767–772, September 1981.

A number of studies have demonstrated that the liver is an important site of glucagon metabolism.[1–5] However, results from different laboratories and from different experiments carried out in the same laboratory have varied considerably, including the observation that the hepatic extraction of glucagon was at times negative.[2,6,7] One possible explanation for this variability might be related to the heterogeneity of circulating plasma immunoreactive glucagon (IRG),[8–10] since widely differing proportions and absolute concentrations of IRG components are known to occur in normal individuals and animals.[11] On the basis of IRG profiles obtained from samples drawn simultaneously from portal and peripheral vessels, the hepatic extraction of immunoreactive glucagon was shown to be confined to the 3500-dalton fraction.[12] However, this study was not quantitative in that it did not take into account hepatic blood flow. In a second study[2] the effects of arginine plus cholecystokinin-pancreozymin infusion on the hepatic extraction of insulin and glucagon and hepatic glucose output in dogs was assessed, although the hepatic extraction of the molecular forms of circulating IRG forms was not quantitated. The following study was therefore undertaken to quantitate the hepatic extraction of circulating IRG components. Chromatographic analysis of the samples derived from seven previously reported experiments[2] and two additional dogs formed the basis of this investigation.

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MATERIALS AND METHODS

After an overnight fast, laparotomy was carried out on nine mongrel dogs (17–25 kg) under pentobarbital (30 mg/kg) anesthesia. Electromagnetic flow probes of appropriate size were placed on the exposed and stripped portal vein and hepatic artery as previously described.[13,14] The portal vein probe was positioned 2 cm inferior to the bifurcation of the common portal trunk and the hepatic artery probe was placed 3 cm from its origin from the celiac trunk. The gastroduodenal branch of the hepatic artery was ligated to prevent the error inherent in the fact that blood flow in this vessel, although measured as part of the hepatic artery blood flow, bypasses the liver. Analogously, the gastroduodenal vein was ligated because it joins the portal vein close to its bifurcation, thus delivering pancreatic blood to the portal vein at a point above which portal flow measurements are made. Before each experiment, flow probes were calibrated by in vitro timed measurements of blood flow and were found to give a linear and accurate response that was unaffected by variation in hemotocrit between 26% and 48%. Polyethylene catheters were inserted into the femoral artery, portal vein, and hepatic vein for blood sampling. The tip of the portal vein catheter was located between the flow probe and the

portal bifurcation. The hepatic vein catheter was inserted through an abdominal route, and advanced 1 cm into the left common hepatic vein. Blood samples for glucagon were obtained simultaneously from the portal vein, femoral artery, and left common hepatic vein. Measurements of blood flow were obtained immediately before each sampling time.

Following a 60-min equilibration period after surgery, blood samples were obtained at −30, −20, −10, and −5 min. At the zero time point, an i.v. infusion of arginine (0.33 g/min in a 10% solution) was started. After 10 min of arginine infusion, 75 Ivy dog units of cholecystokinin (CCK) and 300 Crick, Haper, and Raper units of pancreozymin (PZ) (a gift from the gastrointestinal hormone research unit at the Karolinska Institute, Stockholm, Sweden) was infused with arginine for a further 20 min. Blood samples were drawn at 2, 5, 10, 15, 20, and 30 min after starting the infusion. After 30 min (10 min arginine alone and 20 min arginine plus CCK-PZ), the infusion was stopped and sampling continued every 10 min for an additional 90 min. CCK-PZ were added to the infusion to produce a sustained high hormone release over a prolonged period. Saline (0.9%) was infused into a leg vein to compensate for blood sampling losses. Blood pressure, continuously monitored throughout the study, was stable. Samples for glucagon were collected on ice into tubes containing 500 Kallikrein U/ml Trasylol (FBA Pharmaceuticals, Inc., New York) and EDTA (1.2 mg/ml). Blood flow measurements were corrected to plasma flow by means of hematocrits measured every 30 min. Hepatic vein plasma flow was calculated from the sum of the portal vein and hepatic artery plasma flows. By multiplying plasma concentration of glucagon by the plasma flow in respective vessels, the amount of plasma IRG or IRG components entering and leaving the liver were calculated and the hepatic extraction of whole plasma IRG and the various IRG components were thereby derived.

Immunoassay and chromatographic procedures. IRG was measured by a double antibody radioimmunoassay using 30K antiserum as previously described.[9] The sensitivity of the assay was 3–4 pg/tube. Gel filtration was carried out on simultaneously drawn portal vein, hepatic artery, and hepatic vein plasma samples taken from each animal at baseline, 5–10 min (arginine stimulation), 20–25 min (arginine plus CCK/PZ stimulation), and 70–100 min (recovery period). During each of the four experimental periods, plasma glucagon levels were at steady state.[2] In the baseline period, these samples were derived from pooling of baseline samples, and in the recovery period by pooling 70–100-min samples. In the arginine period (5–15min), four of the nine samples chromatographed were drawn at 5 min, four at 10 min, and one derived from pooling 5- and 10-min samples. In the arginine plus CCK-PZ period (20–25 min), four of the samples were 20-min samples, four were 25-min samples, and one a pooled 20- and 25-min sample. Plasma (0.1–2 ml) was applied to 50 × 1-cm Biogel P-30 columns (Bio-Rad Laboratories, Richmond, California) and eluted under gravity at room temperature with a 0.2 M glycine buffer (pH 8.8) containing 0.25% human serum albumin, 1% lamb serum, and 500 KU/ml of Trasylol. One-milliliter fractions were collected, and the total volume was assayed. Four peaks were identified according to their molecular size:[8–12] > 40,000 daltons (peak A); ≈ 9000 daltons (peak B); 3500 daltons (peak C); <2000 daltons (peak D). The 3500-dalton (biologically active) IRG component was invariably detected, comprising the majority of IRG present. The other three fractions were variably detected, often small and sometimes absent. The total amount of IRG that was fractionated on each column was measured by assay of the particular plasma sample at the time of assay of the column fractions. Recovery of IRG applied to the columns was 86 ± 1.8%, based on 99 column separations. Fractionation could not be performed in 9 of the orginal 108 samples due to inadequate volumes, damage, or loss during laboratory processing. To permit comparison of IRG components from different columns, the IRG levels recovered in each fraction in individual column separations were corrected for under- or over-recovery by adjusting the values to 100%. This correction assumes that there is no selective loss or over-recovery of any particular IRG component.

Statistical methods. Results are expressed as means ± SEM. The statistical significance of difference of group means was assessed by the Student's paired *t* test, P values of less than 0.05 being significant.

RESULTS

The summarized data obtained from the column fractionations are shown in Table 1 and representative elution profiles from the portal vein, hepatic artery, and hepatic vein during the four periods of study in one animal are illustrated in Figure 1.

Hepatic extraction of whole plasma IRG, based on the mean extraction values in each of the four experimental periods, was 25.2 ± 2.5%. Analysis of the hepatic extraction of the four IRG components separated by gel filtration revealed variable and quantitatively insignificant extraction of components other than the 3500 mol. wt. (C peak) component. These were between −3 and +3 ng/min and in most cases close to 0 (Table 1). However, due to the low IRG values in peaks other than the 3500 mol. wt. peak and the variability in this region of the standard curve, the percentage extraction was highly variable based on small quantitative differences in the IRG recovered in these fractions in each of the three vessels. There were no consistent positive or negative extractions of these non-3500 mol. wt. IRG fractions in any individual animal or the group as a whole. Thus, for example, dog 1 had extractions of the void volume (A) peak of 60%, −48%, 100%, and 52% in the four periods, respectively; dog 2, −71%, −2%, −503%, and +23% respectively; dog 3, −64%, +38%, +42%, and +100%, respectively. All these values represented very small absolute positive or negative extractions. The same was true for the B and D peak fractions. The extraction of the 3500-dalton component was consistently positive (30.2 ± 5.7%, 39.6 ± 7.3%, 21.9 ± 6.5%, and 42.2 ± 3.8%), in successive periods, representing 10, 70, 100, and 17 ng/min, respectively. A single C peak extraction value in one animal was negative at −8%, at the 20-min time point. This value accounted for the range of extraction values of −8% to +59%, as shown in Table 1. The mean 3500 mol. wt. glucagon extraction value for all 33 samples derived from the means of the four periods was 33.1 ± 3.2%. Comparison of the extraction of whole plasma IRG with that of the 3500-dalton component is shown in Table 2. Considering all values combined, whole plasma IRG extraction was significantly lower than that of the 3500-dalton component, 25.2 ± 2.5

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TABLE 1
Mean portal vein, femoral artery, and hepatic vein levels of whole plasma IRG and individual plasma IRG components. The hepatic delivery and extraction of these components during the four experimental periods in nine dogs is shown

	Baseline					Arginine (5–10 min)				
	IRG (pg/ml)			Delivery (ng/min)	Extraction (%)	IRG (pg/ml)			Delivery (pg/ml)	Extraction (%)
	PV	HA	HV			PV	HA	HV		
Whole plasma										
M ± SEM	164 ± 14	117 ± 21	117 ± 11	51.5 ± 9.4	18.3 ± 5.9	531 ± 172	232 ± 42	286 ± 78	187.1 ± 80.1	31.0 ± 3.9
Range	(109–220)	(49–217)	(80–170)	(18.5–88.3)	(−8 → 38)	(203–1720)	(94–466)	(124–808)	(70–738)	(1–45)
"A" Peak	23 ± 3	25 ± 8	27 ± 4	7.2 ± 1.2	−23.5 ± 19.1	27 ± 10	34 ± 7	34 ± 9	11.8 ± 3.5	−27.2 ± 49.1
	(4–32)	(12–79)	(6–44)	(3.1–13.7)	(−24 → 60)	(0–73)	(0–57)	(0–87)	(0–31)	(−304 → 100)
"B" Peak	8 ± 4	15 ± 6	10 ± 4	4 ± 1.9	−2.9 ± 11.1	5 ± 4	22 ± 9	9 ± 4	4.1 ± 1.6	29.8 ± 18.2
	(0–26)	(0–48)	(0–21)	(0–11.3)	(−74 → 35)	(0–25)	(0–59)	(0–27)	(0–10)	(−5 → 100)
"C" Peak	119 ± 14	59 ± 10	66 ± 11	31.3 ± 6.7	30.2 ± 5.7	495 ± 167	164 ± 44	231 ± 82	170 ± 78	39.6 ± 7.3
	(80–196)	(18–109)	(37–142)	(12.2–35.4)	(7–47)	(167–1644)	(47–449)	(70–768)	(30–705)	(18–76)
"D" Peak	14 ± 5	17 ± 5	14 ± 5	4.3 ± 1.1	11 ± 13.6	2 ± 2	13 ± 6	13 ± 8	1.7 ± 0.7	−34.3 ± 81.3
	(0–37)	(0–39)	(0–34)	(0–7.8)	(−18 → 100)	(0–12)	(0–47)	(0–62)	(0–5.5)	(−511 → 100)
Plasma flow	211 ± 30	129 ± 12	340 ± 41			248 ± 36	119 ± 19	376 ± 43		
(ml/min)	(102–341)	(71–179)	(173–520)			(101–402)	(74–193)	(175–562)		
Column	94 ± 5	94 ± 6	84 ± 6			85 ± 8	83 ± 3	82 ± 9		
recovery (%)	(79–112)	(76–112)	(58–104)			(55–113)	(58–124)	(66–132)		

	Arginine—CCK/PZ (20–25 min)					Recovery (70–100 min)				
	IRG			Delivery (ng/min)	Extraction (%)	IRG			Delivery (ng/min)	Extraction (%)
	PV	HA	HV			PV	HA	HV		
Whole plasma										
M ± SEM	1275 ± 107	706 ± 90	861 ± 91	526 ± 65.5	22 ± 5.8	192 ± 16	135 ± 21	127 ± 12	57 ± 9.3	31.1 ± 2.3
Range	(900–1760)	(159–990)	(450–1334)	(302–897)	(−5 → 53)	(144–310)	(56–230)	(70–148)	(25–84)	(21–39)
"A" Peak	18 ± 9	17 ± 5	15 ± 7	8.5 ± 3.2	−34.2 ± 64.5	22 ± 8	25 ± 4	20 ± 7	7 ± 1.4	0 ± 39
	(0–66)	(0–39)	(0–50)	(0–25.6)	(−503 → 100)	(0–69)	(8–39)	(0–52)	(2.2–14.9)	(−245 → 100)
"B" Peak	7 ± 5	19 ± 8	8 ± 5	6.1 ± 2.8	20.2 ± 15.2	17 ± 7	9 ± 4	10 ± 4	6.0 ± 3.1	0 ± 29.2
	(0–34)	(0–58)	(0–37)	(0–23.9)	−18 → 100)	(0–63)	(0–39)	(0–23)	(0–26.4)	(−175 → 100)
"C" Peak	1250 ± 109	662 ± 85	836 ± 94	511 ± 65.5	21.9 ± 6.5	153 ± 26	79 ± 20	76 ± 16	40.3 ± 8.2	42.2 ± 3.8
	(845–1765)	(138–956)	(629–1338)	(229–900)	(−8 → 59)	(89–290)	(18–128)	(26–170)	(18–77)	(30–65)
"D" Peak	0	8 ± 4	3 ± 3	1.1 ± 0.7	−53 ± 79	13 ± 4	26 ± 7	14 ± 4	5.1 ± 1.2	−16 ± 44
	—	(0–30)	(0–30)	(0–5.2)	(−676 → 100)	(0–33)	(0–56)	(0–32)	(0–10.4)	(−297 → 100)
Plasma flow	339 ± 38	137 ± 13	476 ± 48			210 ± 37	108 ± 10	319 ± 45		
(ml/min)	(228–590)	(76–198)	(315–764)			(100–381)	(58–160)	(179–541)		
Column	87 ± 5	91 ± 6	91 ± 3			85 ± 6	88 ± 5	85 ± 5		
recovery (%)	(66–114)	(59–105)	(71–101)			(61–104)	(57–102)	(63–100)		

PV, portal vein; FA, femoral artery; HV, hepatic vein; delivery, hepatic IRG delivery.
Values are means ± SEM. Values in parentheses are ranges.

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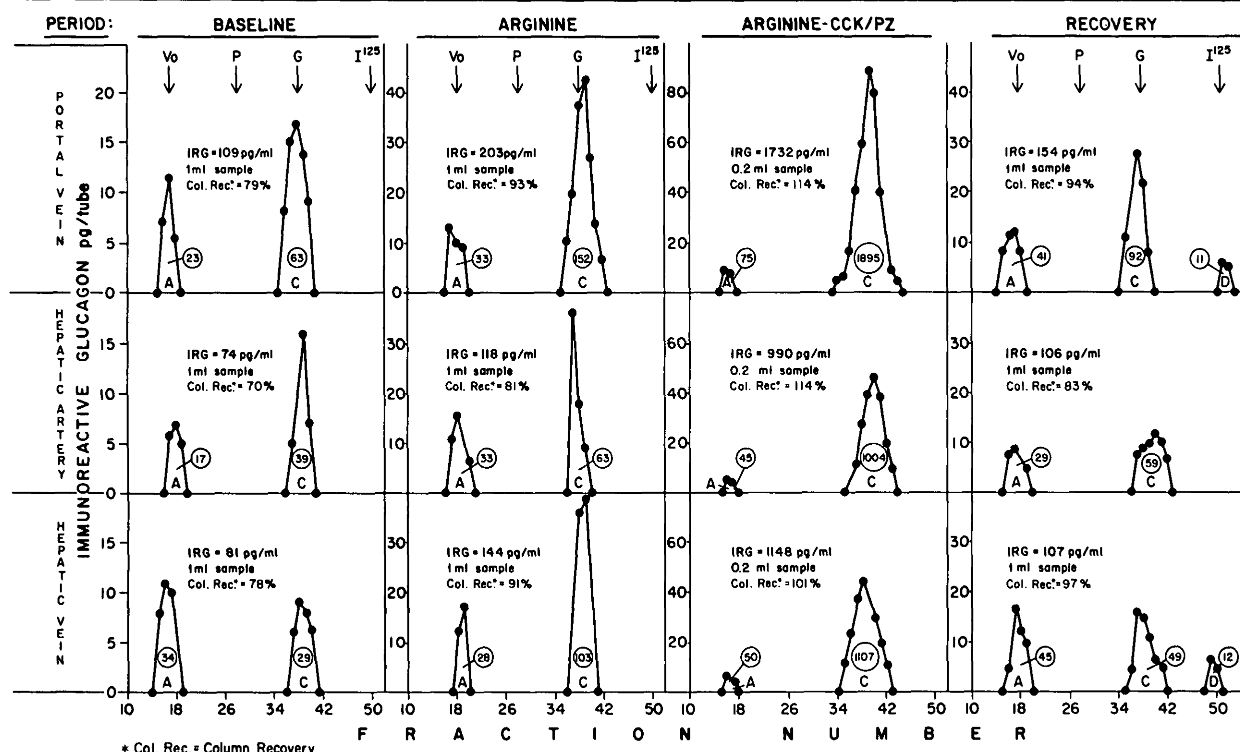

FIGURE 1. Chromatographic profiles from a representative dog taken during the baseline period, arginine infusion (5–10 min), arginine plus cholecystokinin-pancreozymin infusion (20–25 min), and recovery period (70–100 min). At each time point, simultaneously obtained samples from portal vein, hepatic artery, and hepatic vein were eluted under gravity on 1 × 50-cm biogel P-30 columns. One-milliliter samples were fractionated except in the period of maximal stimulation (arginine + CCK/PZ), when 0.2 ml samples were fractionated. All columns were calibrated with ^{125}I rabbit γ globulin (160,000 mol. wt. V°), ^{131}I pork proinsulin (9000-mol. wt. P), ^{125}I glucagon (3500 mol. wt. G), and sodium ^{125}I The encircled numerals refer to the absolute amount of immunoreactive glucagon (pg/ml) detected in each fraction. Note the difference in scale of IRG in different samples. The 3500-mol. wt. (C peak) component comprised the major peak particularly during the two periods of stimulation, with the void volume fraction (A peak) generally comprising somewhat less and occassionally being absent. The amounts of the other two IRG components present were often small, variable, and sometimes absent, as was the case for the 9000-mol. wt. (B peak) component in this dog at all time points and the < 2000-mol. wt. (D peak) component in all but the recovery period.

versus 33.1 ± 3.2 ($P < 0.001$). Further analysis showed that whole plasma IRG extraction was less than that of the 3500-dalton component at each time point during the experiment, except during periods when glucagon secretion was stimulated. At these times, the 3500-dalton component comprised virtually all the circulating IRG and extraction of whole plasma IRG did not differ from that of the 3500-dalton fraction. This relationship is clearly present during the 5–10-min period following arginine stimulation alone, when the non-3500-dalton components comprised between 10% and 25% of total IRG, but it is even more striking at the point of maximal stimulation following arginine plus CCK/PZ (20–25 min), when non-3500-dalton material contributed less than 5% to whole plasma IRG.

TABLE 2
Comparison of hepatic extraction of whole plasma IRG with that of the 3500-dalton IRG fraction

	N	Whole plasma IRG extraction	3500-dalton IRG extraction	P Value
All samples	33	25.2 ± 2.5	33.1 ± 3.2	<0.001
Baseline period	8	18.3 ± 5.9	30.2 ± 5.7	<0.05
Arginine stimulation period (5–10 min)	8	31.0 ± 3.9	39.6 ± 7.3	NS
Arginine + CCK/PZ (20–25 min)	9	22.0 ± 5.8	21.9 ± 6.5	NS
Recovery period (70–100 min)	8	31.1 ± 2.3	42.2 ± 3.8	<0.05

DISCUSSION

Based on work in a number of species, including man, the liver is known to extract 40–60% of the insulin delivered to it.[15,16] In addition, the liver has been shown to extract 30% of somatostatin.[17]

However, it has been claimed that the liver is not a catabolic site of any consequence for glucagon, the other major pancreatic islet peptide,[18,19] and this organ is not generally believed to be important for glucagon clearance. This study, however, strongly supports previous indications[1–5,20,21] that the liver is an important site of glucagon metabolism. Hepatic extraction of whole plasma IRG was 25%, similar to the 23% reported by Jaspan et al.,[1] and the 19% extraction obtained by Röjdmark et al.[2] in the same species. The hepatic extraction values obtained by direct assay of IRG in unfractionated plasma (whole plasma IRG), as well as those of the 3500-dalton component, were similar in the baseline period and arginine plus CCK/PZ stimulation period, despite a 10-fold increase in whole plasma glucagon delivery (51–526 ng/min) and a > 15-fold increase in 3500-dalton IRG delivery (31–511 ng/min). It had previously been

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suggested that hepatic extraction of glucagon was nonsaturable in the range of plasma values extending into the pathologic range (1–2 ng/ml, corresponding to hepatic delivery rates of 700–1000 ng/min).[6] A recent study has extended this observation, indicating that, unlike insulin extraction by the liver,[15,22] hepatic glucagon extraction is nonsaturable to levels extending into the pharmacologic range (plasma levels of 6 ng/ml, corresponding to hepatic delivery of 2000 ng/min). However, it was found that the extraction process is ultimately saturable at a plasma glucagon level somewhere between 6 and 20 ng/ml.[1] The data obtained in this study are consistent with nonsaturability of hepatic glucagon extraction, since there is no significant difference between the extraction in the baseline period (with glucagon delivery of 31 ng/min) and the arginine + CCK-PZ period (with glucagon delivery of 511 ng/min) ($P = 0.15$). However, it is possible that the hepatic glucagon extraction did saturate at some point between period 2 and period 3, when the hepatic glucagon delivery increased from 179 to 511 ng/min, as suggested by the decrease in extraction from 39.6 ± 7.3% (period 2) to 21.9 ± 6.5% (period 3), although this difference is insignificant ($P = 0.16$).

The results demonstrate that the hepatic removal of glucagon is specific for the biologically active 3500-dalton component,[6] as none of the three other plasma IRG fractions was consistently extracted during their passage through the liver. The mean hepatic extraction of the 3500-dalton glucagon component was 33%, which was significantly higher than that of whole plasma IRG (25%). The lower extraction of whole plasma IRG compared with the 3500-dalton component is thus dependent on the presence of various IRG components in plasma that are not metabolized by the liver. As the proportion of non-3500-dalton IRG components is greater under fasting conditions than during stimulation of glucagon secretion, this may be an important factor accounting for quantitative differences in glucagon extraction that have been reported under various physiologic conditions. Thus, depending on the amount of 3500-mol. wt. glucagon present in any particular plasma at any specific time, the percentage of glucagon extracted by the liver may vary considerably. Since plasma glucagon is known to be heterogenous with highly variable proportions of the various circulating IRG components, this may account for the large differences in hepatic glucagon extraction reported by different laboratories.

If hepatic glucagon uptake and degradation are tightly coupled to initial receptor binding, as has been shown for insulin by Terris and Steiner,[23,24] these results suggest that binding to hepatic glucagon receptors may be a property of some but not all of the IRG components. The findings of Rigopoulou et al.[25] are consistent with this notion. These investigators isolated a 9000-dalton IRG fraction from canine pancreas which they termed large glucagon immunoreactivity (LGI). This preparation had no biologic activity, as assessed by its glycogenolytic activity in a perfused rat liver system. Srikant and co-workers[26] also demonstrated that the 9000-dalton IRG component derived from both pancreas and gastric fundus had neither glycogenolytic activity nor adenylate cyclase stimulation activity and only showed minimal displacement of ^{125}I glucagon from rat liver membranes. On the other hand, these investigators were able to show that a 65,000-dalton IRG component derived from the gastric fundus of the dog had equivalent biologic and membrane binding activity to 3500-dalton glucagon. In this regard, Recant et al. studied four plasma IRG fractions derived from glucagonoma patients.[27] They reported equal immunoreactivity and parallel dilution curves with 3500-dalton glucagon standard in all four peaks. The void volume, 3500-dalton, and ≈ 2000-dalton peaks all exhibited biologic activity, as assessed by stimulation of cAMP formation in isolated rat hepatocytes. Their biologic/immunologic activity ratios were approximately 1. The 9000-dalton component, however, appeared to be 25–33% as biologically active as 3500-dalton glucagon, with a biologic to immunologic activity ratio of 0.2–0.3.

The biologic significance of the circulating IRG components other than the 3500-dalton glucagon remains uncertain. Although much remains to be learned about the structure of the larger molecular weight components in plasma, recent studies have considerably enhanced our understanding of the origin and structure of larger molecular weight IRG peptides derived from pancreas and gut. Thus, Tager and Markese[28] and Moody and co-workers[29] have shown that the complete glucagon structure is present in the larger IRG peptides derived from both pancreas and intestine as well as in the 9000-dalton pancreatic peptide, which was postulated[28] to be similar to the ≈ 9000-dalton IRG component that circulates in the plasma of humans and rats in renal failure. Although these peptides are derived from a common gene product, their immunologic and biologic activity may vary, since they contain extensions at either their COOH-terminus or NH_2 terminus or both. Based on the differences in biologic activity and membrane binding of tissue and circulating IRG peptides, it is attractive to speculate that extensions of the basic glucagon sequence such as the N-terminus extension in the 9000-dalton component interfere with hepatic receptor binding and subsequent degradation of the peptide.

The demonstration of selective hepatic degradation of 3500-dalton immunoreactive glucagon has important implications for understanding structure-function relationships of IRG-related peptides.

These findings may also have relevance in relation to mechanisms of hepatic hormone metabolism. Since receptor binding is known to be a saturable process, the differences between the saturability of hepatic insulin extraction on the one hand[15,22] and nonsaturability of hepatic glucagon metabolism on the other[1,6] are noteworthy. Thus, these observations might be interpreted to suggest that while hepatic insulin degradation is dependent on initial receptor binding,[23,24] hepatic glucagon degradation is not dependent on prior receptor binding, or alternatively occurs by more than one mechanism, one requiring receptor binding and the other(s) not. Hepatic somatostatin receptors have not been demonstrated and in this regard, the recent observation that hepatic somatostatin metabolism is a nonsaturable process[17] is consistent with this notion.

In conclusion, an appreciation of the hepatic extraction of glucagon is necessary for the interpretation of data related to biologic activity of glucagon in the liver as well as for estimations of hepatic exposure to glucagon and of pancreatic glucagon secretion rates based on peripheral IRG measurements.

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