Clearance of Immunoreactive Somatostatin by Perfused Rat Liver

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ABSTRACT Other investigators have demonstrated that concentrations of immunoreactive somatostatin (IRS) are higher in blood from the hepatic portal vein or its tributaries than in blood from the hepatic or peripheral systemic veins of man and animals. This suggests that there is hepatic extraction of IRS from the portal system in vivo. In the rat, portal vein plasma IRS is reported to be heterogeneous and to contain, in part, a 1,600 mol wt form of IRS which is immunochemically similar to synthetic somatostatin and not significantly bound to high molecular weight plasma protein. Our study was undertaken to determine directly whether unbound synthetic cyclic somatostatin was cleared by the rat liver perfused through the hepatic portal vein in vitro with a recirculating, plasma-free, erythrocyte-containing perfusate.

At 37°C and pH 7.40, perfusate IRS, at initial concenrations (1,728 pg/ml) within the range previously reported in rat portal venous blood, was removed by the liver at a rate commensurate with first-order kinetics. Hepatic clearance was 0.84±0.04 ml/min per g post-perfusion wet weight (SE). Hepatic extraction was 36±2%, and $t_{1/2}$ was 20.0±1.3 min. Recovery of IRS from the perfusate without the liver was >85%, excluding significant degradation by the medium. Clearance, extraction, and $t_{1/2}$ of IRS were not changed by an unphysiologic IRS concentration (621,500 pg/ml), or by pharmacologic concentrations of insulin (8.2 μM) or glucagon (2.9 μM).

The $t_{1/2}$ was prolonged significantly to 28.2±1.9 and 45.6±4.7 min during perfusions at liver temperatures of 25° and 16°C, respectively. At 37°C, the $t_{1/2}$ was also significantly increased to 28.7±3.2 and 24.2±1.1 min at perfusate pH 7.06 and 6.78, respectively.

These studies indicate that the rat liver clears unbound IRS from the perfusate by a first-order kinetic process that is (*a*) unsaturable at pharmacologic concentrations, (*b*) temperature-sensitive and, to a lesser extent, influenced by lowered pH, and (*c*) not affected by insulin and glucagon. The liver would appear to play an important role in the metabolism of the 1,600 mol wt form of somatostatin. Clearance of endogenous IRS by the liver should be considered in the interpretation of IRS concentrations in the peripheral systemic veins.

INTRODUCTION

Concentrations of immunoreactive somatostatin (IRS)[1] in serum or plasma from man (1) and animals (2–7) are higher in the hepatic portal vein or its major tributaries than in simultaneous samples drawn from the hepatic or peripheral systemic veins. Although the absolute concentrations of IRS are lower if the sample is extracted before the assay (~20–600 pg/ml) (1, 2, 4) than not (~300–2,000 pg/ml) (3, 5), the values suggest that hepatic extraction of IRS might account for the negative transhepatic gradients that have also been shown previously for insulin (8) and glucagon (9, 10). However, definitive proof of IRS uptake requires measurements of total hepatic blood flow and transit time with careful attention to the manner and site of sampling within the vessels (8, 9). These factors were not reported in most of these studies (1–7), but were done in recent experiments in the conscious dog that confirmed hepatic extraction of IRS (11). The demonstration of hepatic uptake in vitro would: (*a*) confirm in vivo studies, (*b*) indicate that the liver is an important site for clearance of portal vein somatostatin derived by release from the pancreas and gut (3, 5, 12) or

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[1] *Abbreviations used in this paper*: IRS, immunoreactive somatostatin; KHB, Krebs-Henseleit bicarbonate buffer.

possibly by influx from the systemic circulation, and (*c*) be in keeping with our previous finding of an inhibition of glucagon-stimulated glycogenolysis in perfused rat liver at pharmacologic somatostatin concentrations (13). No in vitro studies on hepatic metabolism of somatostatin have been published at the time of writing this report.

In the fasted dog, circulating 1,600-mol wt IRS is bound predominately to plasma proteins of 150,000–200,000 mol wt (6, 14). A significant proportion of the total IRS can however exist in the unbound state after a meal (15). However, there is little evidence that IRS binds to high molecular weight protein in rat plasma (4). Also, rat portal venous plasma IRS appears to be heterogeneous, existing in approximately equal amounts of a 1,600 mol wt form which is similar to synthetic somatostatin and a bigger molecular weight form (4).

The purpose of this study, therefore, was to determine whether synthetic cyclic somatostatin would be cleared in vitro by the isolated rat liver, perfused in situ with a plasma-free medium that would limit degradation of IRS caused by plasma itself (4, 16). Clearance of IRS from the perfusate was found and further experiments were performed to characterize the effects of IRS concentration, temperature, pH, and pharmacologic amounts of insulin and glucagon on the clearance process.

METHODS

Materials

Somatostatin, as the synthetic cyclic form, (Bachem Inc., Torrance, Calif.) was prepared for perfusion as aliquots of 0.2, 2, 20, and 80 μg/ml Krebs-Henseleit bicarbonate buffer (KHB) containing 0.5% human serum albumin, fraction V (Sigma Chemical Co., St. Louis, Mo.) and stored at −20°C. [Tyr^{11}] somatostatin for iodination was purchased from Bachem, Inc. The purity of somatostatin and [Tyr^{11}] somatostatin was verified by high pressure liquid chromatography and amino acid analysis (courtesy of Drs. Desiderio and A. Kang, respectively). Crystalline pork insulin containing 0.001% glucagon (a gift from Dr. R. Chance, Eli Lilly and Company, Indianapolis, Ind.) was dissolved in a small volume of 0.01 N HCl, and diluted further with KHB-0.5% human albumin and 0.1 N NAOH to final concentration 3.6 mg/ml, pH 7. Crystalline beef-pork glucagon, containing 47 μU insulin/mg (a gift from Dr. R. Hoseley, Eli Lilly Laboratories) was diluted in 0.005 N HCl at a concentration of 1.4 mg/ml. The insulin and glucagon aliquots contained no detectable IRS when tested in the immunoassay (see below) and were stored at −20°C until used for perfusion within 3 d of preparation. Bovine albumin fraction V (Miles Laboratories, Inc., Elkhart, Ind.) was dialysed as a 10% solution against 10 changes of 5.5 litres KHB at 4°C for 72 h. The albumin concentration was checked with the Technicon Auto-Analyzer (Technicon Instruments Corp., Tarrytown, N. Y.) and aliquots were stored at −20°C until appropriately diluted with KHB before perfusion. Sephadex G-25 (f) was from Sigma Chemical Co., and the column was from Pharmacia Fine Chemicals, Piscataway, N. J. L(+)-Lactic Acid was from Sigma Chemical Co. and D-glucose was from Fisher Scientific Co., Pittsburgh, Pa. All other chemicals were of analytical reagent grade and purchased from commercial suppliers.

Animals

Liver donors were male Wistar rats, purchased from Harlan Laboratories, Indianapolis, Ind., and maintained on standard rat chow and water ad lib. The animals were deprived of food but not water for 48–50 h before killing, when the average body weight was 101 g (range 80–135) and the average postperfusion wet liver weight was 3.4 g (range 2.7–4.5 g). Rats were anesthetized for cannulation procedures with 0.1–0.2 ml sodium pentobarbital (40 mg/ml distilled water) given by i.p. injection.

Liver perfusion technique

Perfusate consisted of 3–4-wk-old human erythrocytes from outdated donor blood washed three times with 2–3 vol of chilled physiologic saline, then twice with chilled KHB 0.1% bovine albumin (17), and finally, suspended in KHB containing 3.5% bovine albumin and 5.5 mM glucose to a hematocrit of 8% (18). The volume of perfusate used to prime the circuit before cannulation of the liver was 135 ml in each experiment. The pH was adjusted (using a glass electrode) to 7.40 with 1 M sodium bicarbonate after equilibration in 95%: 5%, O_2: CO_2 for 20–30 min recirculation at 37°C, or 24–26°C as described below. The recirculating perfusion system of Hems et al. (19) that we previously used to study insulin metabolism (18) was modified by pumping (rather than delivering by a hydrostatic head of pressure) the perfusate from the oxygenator through each liver by means of an infusion pump (model 2100, Extracorporeal Medical Specialties, Inc., King of Prussia, Pa.). During cannulation of the portal vein and inferior vena cava, the flow rate was set at 1.0 ml/min. Once the cannulae were secured and the flow through the liver established (<4 min), the flow rate was increased to 8.0 ml/min, this time point being designated as the start of perfusion. The effluent was discarded for ~5 min perfusion without recirculation until the level of perfusate in the reservoir reached a predetermined 85-ml total vol within the circuit. About 50 ml perfusate was therefore used in the liver washout before recirculating perfusion began. The perfusate in the reservoir was recirculated at 55 ml/min by means of a roller-type pump (model MHRE 200, Watson-Marlow Ltd., Cornwall, England).

In several experiments, the perfusate pressure was measured between the pump and the liver by a pressure transducer (model P23ID, Gould Inc., Medical Products Div., Oxnard, Calif.) connected to a physiologic recorder (Dynograph model R511A, Beckman Instruments, Inc., Shiller Park, Ill.).

In preliminary experiments to test metabolic function (lactate gluconeogenesis [19]) of livers perfused under these conditions with glucose-free perfusate at 37°C and pH 7.40, neutral lactate (1,200 μmol) was added 28 min after the start of perfusion and glucose samples (0.4 ml) were collected from the reservoir at 30 min and every 15 min thereafter for 1 h. Lactate was not added in control studies. At the end of these, and all subsequent perfusions (see below), samples of influx and effluent were collected for blood-gas analyses.

Protocol for somatostatin perfusions

Perfusion at 37°C, pH 7.40. Aliquots of somatostatin (see Methods) were added 30 min after the start of perfusion, thereby allowing a 25-min equilibration period with recirculating perfusate. In certain experiments, insulin, ~5.0 mg

per perfusion or glucagon 1.4 mg per perfusion (see Methods) were added 5 min before somatostatin to obtain, at the time of somatostatin addition, estimated levels of 10 μM for insulin and 5 μM for glucagon. Samples (0.8 ml) for IRS assay were removed from the reservoir 1 min before and for 1 h after addition of somatostatin at the times designated in the figures and tables.

To exclude the presence of IRS-degrading material in the perfusate during liver perfusion with low concentrations (0.2 μg bolus) of IRS, 1 ml medium remaining at the end of perfusion was incubated in duplicate in test tubes for 1 h with ~1 ng fresh somatostatin at 37° and 4°C, to act as a control. For comparison, in some perfusions, 0.16 μg somatostatin was added back to the recirculating medium after 1 h of liver perfusion but with the liver removed; in others, somatostatin was added to fresh perfusate circulating under identical conditions in the circuit without the liver. After perfusion of livers with higher concentrations of IRS, 1 ml aliquots of remaining perfusate were incubated at 4° and 37°C for 1 h without addition of somatostatin to the test tubes.

To exclude binding of IRS to albumin, 80 ml of KHB containing bovine albumin 3.5 g/100 ml, 5.5 mM glucose and 9,300 pg IRS/ml was recirculated at 37°C, pH 7.44 without the liver in the system. After 1 h, 1 ml of medium was applied to a glass-jacketed 29.5 × 2.5 cm Sephadex G-25 (f) column precoated with bovine albumin and equilibrated and eluted at 37°C with 0.1 M sodium phosphate, 0.15 M sodium chloride–0.1% sodium azide, pH 7.4, at a flow rate of 0.5 ml/min. The void volume (62 ml) was marked with dextran blue and fractions (5 ml) were monitored for protein by absorbance at 280 nm and assayed for IRS.

Perfusion at liver temperature of 25° and 16°C. Livers were cannulated and perfused with medium at 24–26°C before recirculation was established. Water from a temperature-controlled water bath was then pumped through a glass jacket surrounding the oxygenator to produce reservoir perfusate temperatures of 23° or 10°C that corresponded, respectively, to actual liver temperatures of 25° or 16°C, which were the result of heat acquired by the perfusate en route to the liver via the pump. Liver temperature was measured directly by a temperature probe placed between the surfaces of adjacent right lobes and connected to a continuously recording telethermometer (model 43 TD, Yellow Springs Instrument Co., Yellow Springs, Ohio). Liver temperatures were stable for 15 min before and 1 h after addition of somatostatin 30 min from the start of perfusion.

Perfusion at lowered pH at 37°C. While being equilibrated with the gas mixture at 37°C, the perfusate pH was reduced with 1 N HCl to 7.10 or 6.80, recorded by means of a glass electrode inserted into the reservoir and connected to a digital readout pH meter calibrated with pH standard solution at 37°C. Livers were perfused at these respective perfusate pH values. Once recirculating perfusion began, the pH of the reservoir medium was monitored at 5–10-min intervals and, because of an initial increase in pH, was adjusted back to ~7.10 or 6.80 with small volumes of 1 N HCl. The desired pH was reached 10 min before somatostatin was added. Recordings of pH with appropriate adjustments were made thereafter at about 15-min intervals and were averaged. The pH values shown in Table II were the means of these averages. Control incubations at 37° and 4°C in test tubes containing 1 ml medium remaining after perfusion supplemented with 1 ng somatostatin were also performed.

Analyses

Samples were kept on ice until centrifuged, supernatant fluids were stored at −20°C and assayed as IRS in <1 mo while concentrations were stable. IRS was measured in triplicate, undiluted for perfusions at low concentrations or at two or three suitable dilutions for higher concentrations, by a modification (20) of a previously characterized immunoassay (21), with synthetic cyclic somatostatin as the standard and the antisomatostatin antibody (sheep B) at a final dilution of 1:150,000. The latter is highly specific and on the basis of cross-reactivity with different analogues and [^{125}I-Tyr^{1}] somatostatin as tracer, reacts with the 4–10th amino acid sequence of the primary structure of somatostatin. [^{125}I-Tyr^{11}]somatostatin, rather than [^{125}I-Tyr^{1}]somatostatin, was used as the tracer because, for reasons that are unclear, the former gave improved binding (20). This was observed by others with an antiserum directed towards the central portion of somatostatin (22). [^{125}I-Tyr^{11}]somatostatin also gave a greater sensitivity (detection limit 10.0 pg/tube or 100 pg/ml perfusate versus 14.3 pg/tube previously reported [21]). Standards like the perfusion samples were prepared in KHB-3.5% bovine albumin −5.5 mM glucose and gave virtually identical displacement curves compared to the standards in conventional buffer (20). The intra-assay coefficient of variation was 8.0% (n = 3). The interassay coefficients of variation in nine consecutive assays were 17 and 19%, respectively, for two internal standards with mean values of 267 and 1,786 pg/ml.

Glucose was measured by the glucose oxidase method, using the Auto-Analyzer (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.). Blood gas analyses were performed with an automatic analyzer (model 175, Corning Medical, Corning Glass Works, Medfield, Mass.). In the experiments with insulin and glucagon, aliquots of perfusate were diluted appropriately with the immunoassay buffers and hormone measurements were made by immunoassay for insulin (23) and glucagon (24).

Calculations

IRS clearance, expressed as milliliter per minute was calculated according to the formula (25): $2.3 \times v/\Delta t \times \log[C1 - C\infty/C2 - C\infty]$. C1 was the initial (5 min) IRS concentration, C2 the final IRS concentration, t, the time interval between C1 and C2 measurements, and C∞ the asymptote approached by C after prolonged perfusion. C∞ was taken as 0 as IRS levels fell below the detection limit of the assay in initial experiments extended for 90 min after addition of low doses of somatostatin. The perfusate volume (V) was calculated from the volume of samples removed and the residual volume pumped out of the circuit plus ~1 ml left behind. V was assumed to remain constant because sampling accounted for about 8% vol loss. Although measured in the erythrocyte-free supernatant fluid, IRS was assumed to be cleared from the whole medium since the hematocrit was 8%. Use of the clearance formula requires that IRS is removed from the medium at a rate that obeys first-order kinetics (25). Therefore, the formula was applied after demonstrating that semi-logarithmic plots of IRS concentration versus time for individual experiments were linear using regression analysis by the least-squares method in a Wang Computer (model WCS 20, Wang Laboratories, Inc., Lowell, Mass.). Clearances were corrected per gram postperfusion wet weight of liver. The formula, hepatic clearance (milliliter per minute per gram) divided by liver flow rate (milliliter per minute per gram) was then used to estimate extraction, i.e., the extent to which the liver extracted IRS in a single passage of perfusate (25). The average flow rate for all the experiments was 2.3 ml/min per g (range 1.8–3.0). The $t_{1/2}$ (minute) of IRS was computed using the formula $t_{1/2} = 0.693/K$, where K (per minute) was the disappearance rate constant or the value obtained for the slope of the line on the semi-log plot.

For studies on glucose formation in the absence or presence of lactate, rates of gluconeogenesis were calculated from the slopes of arithmetic plots of total perfusate glucose (micromoles) versus time (minutes), and corrected per gram liver wet weight.

All values henceforth shown in the text, tables, figures, or their legends are mean±SE, unless otherwise indicated.

Statistical analysis

Student's t test for unpaired samples was used, a P value of <0.05 for differences between means being considered statistically significant.

RESULTS

Metabolic viability and stability of livers. Initial studies to validate the metabolic integrity of livers perfused at average flow rates of 2.3 ml/min per g (range 2.0–2.6) and at pH 7.40, 37°C showed that without added lacetate, livers synthesized glucose at rates of 0.18±0.06 μmol/min per g, (five experiments), produced bile at 74 μl/h per g and consumed oxygen at 2.1±0.2 μmol/min per g, values compatible with previous studies (18, 19).

At flow rates of 2.3 ml/min per g (range 1.9–2.8), addition of 15 mM lactate stimulated glucose formation to maximal rates of 0.74±0.06 μmol/min per g (five experiments) which were linear for 1 h, and were accompanied by a significant ($P < 0.05$) increase in oxygen uptake to 2.8±0.2 μmol/min per g when measured at the end of perfusions. Bile formation was 60±2 μl/h per g.

Addition of increasing concentrations of somatostatin did not significantly influence basal oxygen uptake (Table I). The variability in bile production rate in these experiments is difficult to interpret, since small changes in bile formation may result from mechanical factors that influence bile collection. However, we have previously noted no effect of somatostatin on oxygen uptake or bile production in a nonrecirculating system (13). Basal oxygen uptake fell to 0.58±0.15 μmol/min per g and bile formation to 31±7 μl/h per g at 25°C. At 16°C, oxygen uptake and bile formation were reduced further to 0.28±0.13 μmol/min per g and 12±2 μl/h per g, respectively.

As shown in Table II, reduction of mean perfusate pH from 7.40 to 7.06 did not affect bile formation or oxygen uptake. At mean pH 6.78, oxygen uptake (1.6±0.1 μmol/min per g) was reduced by 20%, which was not statistically different from that at pH 7.40. Bile production also remained unchanged.

The mean pressure of the influx was 6.6 mm Hg (range 5.5 to 9.0 for four experiments) both before and during low (1.7 ng/ml, $n = 1$) or high (20–30 ng/ml, $n = 3$) IRS perfusions. Perfusion pressure remained stable (5.0 and 7.0 mm Hg) before and during two IRS perfusions at 16°C. These pressures are lower than normal portal venous pressure in the rat, reported as 12–14 cm blood (26), or 9–10 mm Hg assuming the specific gravity of blood = 1 and the specific gravity of Hg = 13.6. The absence of an effect in this system differs from the prompt lowering of portal venous pressure (27) or flow (28) after somatostatin infusion in vivo.

Finally, during all studies, livers looked macroscopically intact without patchy areas to suggest malperfusion or infarction. They were not distended, and did not exude perfusate from their surfaces. Wet to dry liver weight ratios after perfusion were within a narrow range (3.27–3.68) in all the various groups of studies.

Exclusion of IRS binding to albumin. On Sephadex G-25 (f) chromatography at 37°C, pH 7.4, IRS that had been incubated with perfusate alone at similar temperature and pH, eluted in a peak corresponding to 2 void vol of the column (124 ml) with a recovery of 46.2% of the applied immunoreactivity. No IRS was detected in the protein peak in the void volume, thereby excluding any significant binding of IRS to bovine albumin under physiologic conditions.

Hepatic clearance of IRS at 37°C, pH 7.40. Before addition of somatostatin to the reservoir, IRS in the perfusate was consistently undetectable. Five minutes after the addition of a 0.2-μg bolus to allow equilibration in the circuit, mean IRS concentration of 1,728 ±139 pg/ml approximated the highest levels of IRS measured in dog plasma (14, 15) or unextracted rat hepatic portal vein serum (3). As shown in Fig. 1, the concentrations fell at a curvilinear rate, which linearised on a semilogarithmic plot with a $t_{1/2}$ of 20.0±1.3 min and a regression coefficient of −0.989±0.003 (Table I). The latter value was significantly greater ($P < 0.001$) than that, −0.961±0.008, computed from conventional arithmetic plots. Hepatic clearance was 0.84±0.04 ml/min per g, from which it was calculated that 36±2% of IRS was extracted from the perfusate in one transhepatic passage (Table I). The recovery of IRS was 93.2±4.5% from the perfusate, which had been incubated for 1 h at 37°C in test tubes after completion of these perfusions. By comparison and in close agreement, 91.0±1.0% of the initial IRS concentration remained 1 h after adding a fresh charge of somatostatin to the circulating medium with the liver removed (Fig. 1). In three separate experiments, 88.0±14.5% remained after 1 h recirculation in fresh medium previously unexposed to liver (Fig. 1). During the course of the initial viability studies as well as in these and the subsequent IRS perfusions, no visible hemolysis was observed in perfusate supernatant fluids, with the exception noted at perfusion pH 6.78. The results show that loss of IRS attributable to the medium, per se, during liver perfusion was small.

Effect of increasing IRS concentration on its

TABLE I

*Effect of Increasing IRS Concentration on Hepatic Clearance of IRS**

Number of perfusions	Rat body weight	Liver weight	Flow rate	Bile formation	Oxygen uptake	Time after addition of somatostatin (*min*)							Regression coefficient	Slope	$t_{1/2}$‡	Hepatic clearance	Hepatic extraction
						5	10	20	30	40	50	60					
	g	*g*	*ml/min per g*	*μl/h per g*	*μmol/min per g*	*IRS (pg/ml)*								*min*$^{-1}$	*min*	*ml/min per g*	%
6	104	3.6	2.3	62	2.0	1,728	1,454	1,025	715	491	380	243	−0.989	−0.0354	20.0	0.84	36
	±7	±0.3	±0.3	±4	±0.1	±139	±123	±111	±50	±43	±28	±28	±0.003	±0.0021	±1.3	±0.04	±2
3	88	2.9	2.8	75	2.4	28,533	21,983	17,510	11,940	7,713	5,540	3,998	−0.994	−0.0379	18.5	1.1	38
	±3	±0.3	±0.3	±8	±0.01	±5,558	±3,063	±2,912	±1,344	±1,073	±673	±1,102	±0.002	±0.0026	±1.2	±0.2	±4
3	92	3.2	2.5	83	1.9	133,333	142,633	99,400	71,167	52,567	38,167	26,167	−0.985	−0.0311	22.3	0.76	30
	±2	±0.1	±0.1	±9	±0.1	±4,539	±17,066	±777	±9,138	±1,417	±3,667	±706	±0.004	±0.0008	±0.6	±0.04	±1
3	105	3.8	2.1	54	2.1	621,500	501,000	356,230	245,933	183,683	132,500	102,333	−0.992	−0.0329	21.3	0.72	34
	±1	±0.2	±0.1	±7	±0.1	±28,862	±54,187	±6,332	±17,512	±18,426	±13,565	±11,560	±0.002	±0.0003	±1.8	±0.05	±3

* 37°C, pH 7.40.

‡ There are no significant differences in $t_{1/2}$, hepatic clearance, or hepatic extraction between any of the four groups of perfusions.

TABLE II
*Effect of pH on Hepatic Clearance of IRS**

No. of perfusions	pH of perfusate	Rat body weight	Liver weight	Flow rate	Bile formation	Oxygen uptake	Time after addition of somatostatin (*min*)							Regression coefficient	Slope	$t_{1/2}$‡	Hepatic clearance§	Hepatic extraction‖
							5	10	20	30	40	50	60					
		g	*g*	*ml/min per g*	*μl/h per g*	*μmol/min per g*	IRS (*pg/ml*)								*min*$^{-1}$	*min*	*ml/min per g*	%
6	7.40	104	3.6	2.3	62	2.0	1,728	1,454	1,025	715	491	380	243	−0.989	−0.0354	20.0[a]	0.84[a]	36[a]
	±0.01	±7	±0.3	±0.3	±4	±0.1	±139	±123	±111	±50	±43	±28	±28	±0.003	±0.0021	±1.3	±0.04	±2
3	7.06	103	3.7	2.1	65	2.3	1,866	1,759	1,521	1,053	769	696	532	−0.983	−0.0247	28.7[b]	0.54[b]	26[b]
	±0.03	±2	±0.1	±0.02	±6	±0.1	±203	±132	±192	±118	±121	±109	±142	±0.003	±0.0027	±3.2	±0.07	±3
6	6.78	107	3.9	2.1	61	1.6	1,598	1,379	1,107	806	610	447	346	−0.990	−0.0289	24.2[c]	0.58[c]	28[c]
	±0.02	±2	±0.1	±0.1	±3	±0.1	±225	±170	±163	±131	±80	±78	±83	±0.004	±0.0013	±1.1	±0.01	±1
1¶	6.82						1,120	1,090	970	1,140	1,220	1,170	1,210	—	—	—	—	—

* 37°C.
‡ For $t_{1/2}$: a vs. b, $P < 0.02$; a vs. c, $P < 0.05$; b vs. c, $P > 0.05$.
§ For hepatic clearance: a vs. b, $P < 0.01$; a vs. c, $P < 0.001$; b vs. c, $P > 0.05$.
‖ For hepatic extraction: a vs. b, $P < 0.01$; a vs. c, $P < 0.01$; b vs. c, $P > 0.05$.
¶ Fresh perfusate without liver.

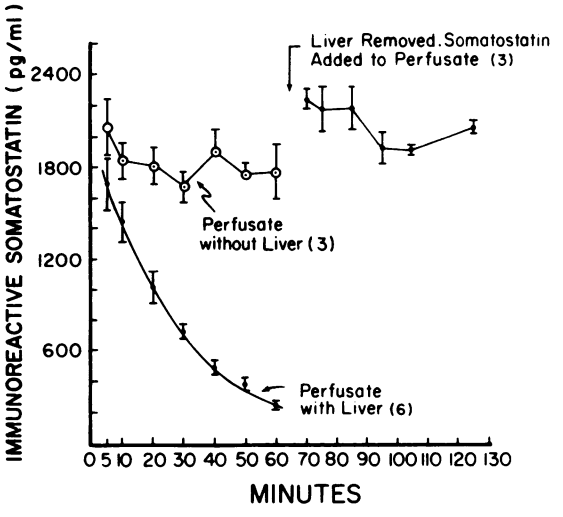

FIGURE 1 Disappearance of IRS from the circulating perfusate during perfusion with and without the liver. A small decrease in concentration occurs in perfusate either previously unexposed to liver or continuing to recirculate after removal of the liver. Number of experiments appear in parentheses.

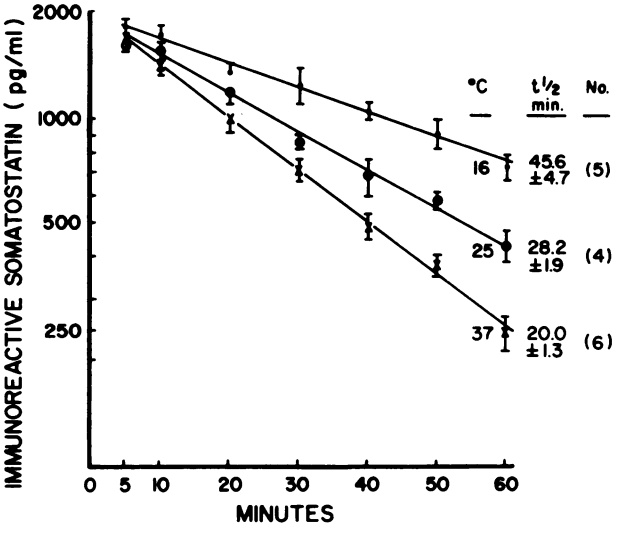

FIGURE 2 Semilogarithmic plots showing effect of temperature on hepatic clearance of IRS from the perfusate. Disappearance rate constants (min^{-1}) were -0.0354 ± 0.0021 at 37°C; -0.0250 ± 0.0019 at 25°, and -0.0158 ± 0.0015 at 16°C. Regression coefficients were -0.989 ± 0.003 at 37°C, -0.976 ± 0.010 at 25°C, and -0.958 ± 0.010 at 16°C. Number of experiments are shown in parentheses.

clearance. As shown in Table I, raising the initial mean concentrations to 28,533, 133,333, and 621,500 pg/ml did not significantly affect the $t_{1/2}$ of circulating IRS, its clearance, or the hepatic extraction. Recovery of IRS from the residual perfusate upon further incubation for 1 h at 37°C was 88.5±3.1% and 90.0±3.5% for experiments performed at the two latter concentrations, respectively.

Effect of reduced liver temperature on IRS removed from the perfusate. Fig. 2 illustrates that the mean $t_{1/2}$ at 37°C of 20.0±1.3 min increased to 28.2±1.9 min at 25°C ($P < 0.001$) and to 45.6±4.7 min ($P < 0.001$) at 16°C. Expressed as a percentage of the initial (5 min) value, IRS remaining in the circulating perfusate after 1 h at 37°C was 14±2%, at 25°C was 27±2%, and at 16°C was 41±3%.

Effect of lowered pH on IRS clearance at 37°C. Table II shows that when the mean perfusate pH was adjusted from 7.40 to 7.06, $t_{1/2}$ lengthened from 20.0 ±1.3 to 28.7±3.2 min, hepatic clearance fell from 0.84±0.04 to 0.54±0.08 ml/min per g, and extraction was reduced from 36±2% to 26±3%. These differences, while small, were statistically significant (see footnote to Table II). At perfusate pH 6.78±0.02, no further changes in $t_{1/2}$, hepatic clearance or extraction of IRS occurred. Recovery of IRS from media after perfusions was 90.3±4.5% at pH 7.06 and 82.3±1.5% at pH 6.78. The recoveries at pH 6.78 were significantly lower ($P < 0.05$) than those (93.2 ± 4.5%) in studies at pH 7.40, possibly related to visible slight hemolysis in the medium. However, as shown in Table II, IRS concentrations were stable in circulating perfusate without the liver at pH 6.82, suggesting release of small amounts of degrading material from the liver into the medium at this extreme pH.

Effect of pharmacologic concentrations of insulin and glucagon. Hepatic clearance of IRS was not influenced by high concentrations of insulin or glucagon (Figure 3). Compared to the range of $t_{1/2}$ of IRS alone, (17.2–24.2 min) the $t_{1/2}$ for IRS in two separate experiments with insulin were 19.1 and 16.1 min, and with glucagon 17.6 and 19.4 min. Average hepatic extraction for IRS in these two groups of studies was 37% with insulin and 38% with glucagon. Both were within the normal range for extraction of IRS alone (30–42%). In these four experiments, recovery of IRS in control incubations at 37°C was 92.3±3.6%.

Because insulin and glucagon are themselves degraded by liver (29–31), their concentrations during perfusion were measured. The average immunoreactive insulin concentration when somatostatin was added to the reservoir was 8.2 μM (49 μg/ml), and fell to 6.0 μM at the end of 60 min of perfusion. Likewise, the initial immunoreactive glucagon level was 2.9 μM (10 μg/ml), and 2.0 μM at the end of perfusion. Although the initial concentrations were 82 and 58% of those expected for insulin and glucagon from the estimated amounts of each hormone added, respectively, other in vitro studies with intact rat liver cell preparations suggest these concentrations were high enough to saturate insulin (30) or glucagon receptors (32) by 90%

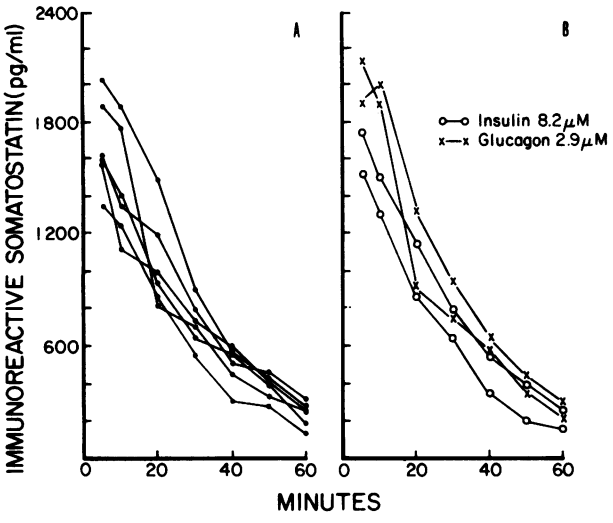

FIGURE 3 Failure of IRS clearance to be affected by the presence (B) or absence (A) of pharmacologic concentrations of insulin and glucagon. Each disappearance curve represents results from one separate liver perfusion.

or more during perfusion, or to compete with insulin or glucagon-degrading enzymes (31).

DISCUSSION

The series of experiments described in this study demonstrate that the rat liver possesses a system which rapidly clears IRS circulating unbound in the perfusate. They also provide direct evidence for a role of the liver in somatostatin metabolism.

At physiologic pH and temperature, hepatic clearance of IRS obeyed first-order kinetics at concentrations within the range of IRS measured in unextracted (3) or extracted (4) rat hepatic portal blood or of unbound IRS in dog portal plasma (14, 15). IRS clearance was not saturable at pharmacologic IRS concentrations (621 ng/ml or 388 nM). The failure to saturate hepatic clearance was not because of a significant amount of degrading material in the medium, because recovery of IRS from the perfusate without the liver was >85%. In contrast, immunoreactive insulin clearance is saturable at even lower molar concentrations in similar liver perfusion systems (18, 29), thus excluding in vitro artifact as a cause for lack of saturability of IRS clearance. Further studies are required to determine whether the high hepatic capacity for IRS clearance is a property of the hepatocyte or Kupffer-cell populations, or both.

The IRS clearance process was less effective after lowering the temperature of the liver and, to a more modest extent, the pH of the perfusate. This temperature sensitivity and pH effect suggests that temperature-dependent transport processes, binding interactions, or enzymatic degradation by cellular proteins are mechanisms that could mediate disappearance of the peptide from the medium. Possible evidence for a binding step in the clearance process includes (*a*) the report of an 80,000 mol wt partly purified "somatostatin-binding protein" in rat liver cytosol (33) and, to a lesser extent, in particulate fractions and, (*b*) the observation that 59% of IRS was removed by the liver at 16°C. The existence of a specific somatostatin-binding protein in rat liver requires independent confirmation. Furthermore, enzymes that are active at cold temperatures, or non-enzymatic degradation, could have played a role in the clearance of IRS at 16°C.

At pH ~6.8, mean hepatic clearance (0.58 ml/min per g) and extraction (28%) were significantly lower than clearance (0.84 ml/min per g) and extraction (36%), respectively, at pH 7.4. These modest differences suggest that the liver still retains a substantial capacity for handling physiologic amounts of IRS at a pH low in the pathophysiologic range. This is possibly because intracellular pH might not have changed significantly (34). The relevance of this experimental observation to circulating IRS levels in diseases characterized by acidosis in man is unknown.

Pharmacologic concentrations of insulin (6–8 μM) or glucagon (2–3 μM) in the perfusate did not impede clearance of IRS. Likewise, high concentrations of somatostatin (8 μM) did not influence insulin clearance in a similar perfusion system (18). Also, glucagon did not affect insulin clearance by perfused rat liver (18, 35). Thus, while insulin, glucagon, and somatostatin affect each other's secretion by the pancreatic islets (36), present evidence suggests that they do not appear to modify one another's clearance by intact liver preparations in vitro. Ishida and co-workers (10) recently reported that exogenous somatostatin infusion modified hepatic extraction of insulin and glucagon in the anesthetized dog. The discrepancy between their observations in vivo and the results in vitro (18) might be due, inter alia, to species differences or to profound effects of somatostatin infusion on hepatic portal venous-blood flow (10, 28) that are not observed in the isolated perfused liver system.

The clearance of IRS by the liver does not appear to be a nonselective type of process because other low molecular mass peptides that are secreted into the hepatic portal vein such as C-peptide (3,000 daltons) from the pancreas or heptadecapeptide gastrin (2,098 daltons) from the stomach are not significantly extracted by perfused rat liver (37, 38). Bovine 1-84 parathyroid hormone, which primarily reaches the liver via the systemic arterial circulation, is specifically cleaved by perfused rat liver to parathyroid hormone fragments that are biologically active and resemble those seen in the peripheral blood (39).

Our experiments characterized the interactions between the isolated rat liver and unbound IRS in the perfusate that simulates circulating IRS in the rat, in which plasma protein binding of IRS is negligible (4). However, in other species, such as the dog, plasma binding of IRS (6) must be considered as an important variable that could influence the in vivo hepatic clearance of the peptide.

From a teleologic viewpoint, the liver probably serves as a major site for inactivation, by degradative catabolism, of somatostatin. The liver has a strategic anatomic location between the somatostatin-rich gut and pancreas and the systemic circulation, into which the unrestricted entry of active somatostatin from the gastrointestinal tract might have excessive ubiquitous effects. Rapid hepatic clearance and catabolism could complement plasma degradation and, in certain species, a neutralizing effect of plasma protein binding as mechanisms to limit the peripheral effects of somatostatin. We have not examined hepatic degradation of somatostatin, but it is highly likely that rather than accumulate unchanged in the liver, the peptide is ultimately cleaved into small fragments. This has been demonstrated with homogenates of brain (40), and in blood (1, 4, 16). Further studies on this point are needed.

The liver might also be a site of biologic action for somatostatin. Clearance of the peptide and the presence of extractable, biologically active somatostatin in rat hepatic portal vein plasma (41) would favor this role, but the evidence in favor of this notion is conflicting. Reports of inhibition by somatostatin of glucagon-stimulated glucose formation by perfused rat liver (13) and isolated hepatocytes (42), and of cyclic AMP released by isolated rat hepatocytes (43) and liver cell plasma membranes (44) must be interpreted cautiously, because the concentrations of somatostatin were unphysiologic, i.e., 20–1,000-fold higher than IRS levels in blood. Similar effects in vitro have not been found by other investigators (45, 46). Presently, therefore, a physiologic action for somatostatin on hepatic metabolism appears unsubstantiated, but pharmacologic effects could still occur.

The very high capacity of the liver to clear the 1,600 mol wt form of somatostatin from the hepatic-portal influx has physiologic and clinical implications in the interpretation of levels as well as the effects of IRS circulating in peripheral venous blood. First, either no significant changes (1) or only relatively small increments in peripheral vein IRS have been measured in normal man (47) following nutrient stimuli that elevate markedly the IRS concentrations in the portal venous systems in animals (3, 5, 14, 15). Preliminary evidence for the existence of a transhepatic IRS gradient in obese patients had been reported recently (1). Second, a higher molecular weight form of IRS ("big IRS") not dissociable by 6 M urea, has been identified (4) in peripheral rat plasma as the dominant species (95%), and has also been found in a small proportion of human sera (16). Because the 1,600 mol wt form and big IRS were found in approximately equal amounts in rat portal blood (4), it is possible that the bigger form was less susceptible to hepatic uptake and degradation and appeared more readily peripherally. In support of this possibility, 1,600 mol wt IRS was very rapidly degraded by human amniotic fluid, but the big IRS in the fluid was not degraded (48). Similar precedents have been established for insulin, which is cleared and degraded more rapidly by the liver than proinsulin (29). Finally, it is conceivable that in certain diseases in man, such as somatostatinoma of the pancreas, the liver may restrict or limit increased amounts of free, biologically active, 1,600 mol wt somatostatin, secreted by these tumors (49) and identified in peripheral blood (49, 50), from entering the systemic circulation unless the primary is large and/or hepatic metastases develop (49, 50).

In conclusion, the perfused rat liver clears IRS in its unbound state from the perfusate by a first-order kinetic process that cannot be saturated at unphysiologic concentrations (388 nM) of the peptide. Clearance is temperature-sensitive, modestly reduced by lowered pH, and not influenced by pharmacologic levels of insulin and glucagon. Further studies are required to characterize the mechanism(s) involved. Hepatic extraction of IRS released from the pancreas and gastrointestinal tract should be considered in evaluating the changes or effects of IRS in peripheral venous blood.

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