Compartmental Modeling of Glucagon Kinetics in the Conscious Dog

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The aim of the present study was to examine glucagon metabolism and distribution using both compartmental-modeling approaches and steady-state organ-balance techniques in conscious, overnight-fasted dogs. Arterial plasma glucose concentrations were clamped at 14 mmol/L with a variable exogenous glucose infusion. Somatostatin was infused to block endogenous secretion of insulin and glucagon. Insulin was replaced intraportally at 2.4 pmol · kg^{-1} · min^{-1} to maintain basal insulin concentrations in the range from 70 ± 4 to 95 ± 12 pmol/L. Glucagon was not given during the control period, but was subsequently infused peripherally in four 1-hour steps of 1.0, 3.0, 6.0, and 3.0 ng · kg^{-1} · min^{-1}. Glucagon levels increased from 0 to 68 ± 6, 195 ± 19, 378 ± 47, and 181 ± 20 ng/mL. Compartmental analysis of glucagon concentrations showed that glucagon was distributed in one compartment with a volume approximately equal to the plasma volume. The metabolic clearance rate of glucagon was 17.6 mL · kg^{-1} · min^{-1}. The liver cleared 24% of glucagon, and the kidneys, 17%.

THE CONCENTRATION of glucagon in plasma is determined by the rate of secretion from α cells of the pancreas or gastrointestinal mucosa, the distribution of hormone between plasma and other body pools, and the rate of irreversible removal of glucagon. A change in any one of these factors can result in altered plasma glucagon concentrations and subsequent changes in substrate metabolism. It is also possible that multiple factors change in such a manner that plasma glucagon levels remain normal even when the underlying hormone unit processes are defective.

In vivo glucagon kinetics have not been completely characterized. Jaspan et al,[1] using an anesthetized dog model, performed the most comprehensive series of experiments examining whole-body glucagon kinetics. Endogenous glucagon and insulin secretion were eliminated by total pancreatectomy or suppressed by somatostatin infusion. Basal replacement doses of insulin were administered, and glucagon was infused at rates varying from 1 to 300 ng · kg^{-1} · min^{-1}. In some experiments, the glucagon infusion was increased in three steps of 45 minutes' duration, whereas in other experiments it was constant for all three 45-minute periods. Blood was sampled from catheters placed in the portal vein, hepatic vein, and femoral artery at 35, 40, and 45 minutes of each period for determination of plasma glucagon levels. Hepatic artery and portal vein blood flow were measured with electromagnetic flow probes. The investigators estimated that the whole-body plasma clearance rate of glucagon was 12.6 ± 0.8 mL · kg^{-1} · min^{-1}. The hepatic clearance rate was 4.2 ± 0.3 mL · kg^{-1} · min^{-1}, and hepatic fractional extraction (FE) of glucagon was 22.5% ± 1.7%.[1] Separate experiments determined that the renal clearance rate was 4.7 ± 0.5 mL · kg^{-1} · min^{-1}, whereas renal FE of glucagon was 43.7% ± 3.9%.[2,3]

The studies described earlier and additional studies[1-7] have provided useful information regarding whole-body and individual-organ clearances of glucagon. However, all experiments were conducted in anesthetized animals immediately following surgical placement of sampling catheters, and some experiments followed even more invasive surgical procedures such as pancreatectomy. It is known that the stress of anesthesia and surgery can alter blood flow to the liver and kidney and the function of these organs,[8] so it is uncertain whether the results truly represent normal glucagon metabolism. In addition, steady-state noncompartmental approaches used to measure plasma clearance rate in the aforementioned studies ignored the useful information that could be gained from transitions from one steady state to another. A separate class of models, compartmental models, have been used to provide a fuller, richer description of kinetics for substances such as glucose and insulin.[9-14] No one has ever used compartmental analysis to identify important parameters of glucagon kinetics such as the number of compartments into which glucagon distributes, the volume of the compartments, and exchange rates between compartments. Determining these parameters of glucagon kinetics will help increase the understanding of the timing of events in the hepatic response to fluctuations of glucagon secretion, such as those seen in the response to feeding or fasting,[15-19] diabetes,[20-22] hypoglycemia,[23-25] trauma,[26] sepsis,[27] and exercise.[28]

MATERIALS AND METHODS

Animal Care and Surgical Procedures

Experiments were performed on mongrel dogs (20 to 24 kg) of either sex that had been fed once daily with a standard diet of Kal Kan beef dinner (Vernon, CA) and Purina Dog Chow (Purina Mills, St Louis, MO; 51% carbohydrate, 31% protein, 11% fat, and 7% fiber by dry weight). Dogs were housed in a surgical facility that met all American Association for the Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by the University Animal Care Committee.

Two weeks before each experiment, a laparotomy was performed with the animal under general anesthesia (sodium pentobarbital 25 mg/kg). Using standard sterile techniques previously described,[29] Silastic catheters (0.03-in ID, Dow Corning, Midland,

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MI) were placed in the jejunal and splenic veins for infusion into the hepatic portal system. Additional Silastic catheters (0.04-in ID) were inserted in the major left common hepatic vein, the portal vein, and the left renal vein for blood sampling. The major left common hepatic vein and the left branch of the portal vein were exposed by retracting the left lateral lobe of liver caudally and the central lobe cephalically. A small opening was made in the left common hepatic vein 2 cm from its exit from the left lateral lobe of the liver using a 14-gauge Angiocath (Monoject, St Louis, MO). The sampling catheter was inserted through the opening, passed retrogradely 1.5 cm, and secured in place with three ties of 5.0 Ticron (Davis & Geck, Danbury, CT). In the same manner, a sampling catheter was inserted into the portal vein through an opening 2 cm from its entrance to the liver and passed retrogradely about 4 cm and secured into place. The left renal vein catheter was inserted through a small opening made at the junction of the vein with the inferior vena cava. The catheter was passed retrogradely past the left gonadal vein to within approximately 1 cm of the hilus of the kidney and then held in place by a purse-string suture placed in the adventitia of the vessel. To ensure that the catheter tips had not migrated from their correct positions during the 2-week recovery period before the experiment, placement of all catheters was verified during autopsy following each study. The gastroduodenal artery was ligated, and Doppler flow probes (Instrument Development Laboratories, Baylor College of Medicine, Houston, TX) were placed around the portal vein and hepatic artery as previously described[30] for measurement of hepatic blood flow. The free ends of the catheters and flow probes were placed in subcutaneous pockets, and the abdominal cavity was closed. A Silastic catheter (0.04-in ID) was also inserted into the left femoral artery for blood sampling using a cut-down procedure. Again, this catheter was placed in a subcutaneous pocket so the skin incision could be closed. Penicillin G (500 kU, Pfizer, New York, NY) was administered postoperatively, and ampicillin (1,000 mg orally, Squibb-Novo, Princeton, NJ) was given with meals for 2 days.

Three days before each experiment, blood was withdrawn to determine hematocrit and leukocyte count. Experiments were performed only on dogs that met the following criteria: (1) leukocyte count less than 18,000/mm^3, (2) hematocrit greater than 35%, (3) a good appetite, and (4) normal stools. Each dog was fasted for 18 hours before the study. On the morning of the experiment, the ends of the catheters were exteriorized through incisions made under local anesthesia (2% lidocaine, Abbott Laboratories, Chicago, IL) and the catheter lumens were flushed with heparinized saline (1 U/mL). Twenty-gauge Angiocaths were inserted percutaneously into cephalic and saphenous veins for infusion of hormones and dyes. Each animal was allowed to rest quietly in a Pavlov harness for 30 minutes before the experiment.

Experimental Procedures

Each experiment (n = 5) consisted of an equilibration period (−120 to −30 minutes), a control period (−30 to 0 minutes), and an experimental period (0 to 240 minutes). Constant infusions of peripheral somatostatin (0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$; Bachem, Torrance, CA) and portal insulin (2.4 $pmol \cdot kg^{-1} \cdot min^{-1}$; Lilly, Indianapolis, IN) were initiated at the start of each experiment. A primed peripheral infusion of glucose was also started, and plasma glucose concentrations were clamped at approximately 14 mmol/L. This glucose level was then maintained for the duration of the experiment. In addition, indocyanine green (Hynson, Westcott and Dunning, Baltimore, MD) and *p*-aminohippurate (Sigma Chemical, St Louis, MO) were infused for determinations of hepatic and renal blood flow.[31,32] Three blood samples were drawn from the femoral artery, portal vein, hepatic vein, and renal vein at 15-minute intervals during the steady-state basal period. The parameters measured in steady state included: (1) plasma glucagon levels in the femoral artery, portal vein, hepatic vein, and renal vein; (2) plasma glucose levels in the femoral artery, portal vein, and hepatic vein; (3) arterial plasma insulin levels; (4) hepatic and renal plasma flow; and (5) hematocrit.

The experimental period was subdivided into four 1-hour segments. At the beginning of each segment, the peripheral infusion of glucagon (Eli Lilly, Indianapolis, IN) was changed to a new rate. At 0 minutes, glucagon infusion began at a basal rate of 1.0 $ng \cdot kg^{-1} \cdot min^{-1}$. The infusion rate was increased to 3.0 $ng \cdot kg^{-1} \cdot min^{-1}$ at 60 minutes, increased to 6.0 $ng \cdot kg^{-1} \cdot min^{-1}$ at 120 minutes, and decreased to 3.0 $ng \cdot kg^{-1} \cdot min^{-1}$ at 180 minutes. The sampling schedule was the same for each 1-hour segment. After the glucagon infusion rate was changed, arterial blood samples were taken after 1, 3, 5, 7, 9, 11, 13, 15, 20, 25, and 30 minutes for determination of arterial plasma glucagon concentrations. Each sample took 15 to 20 seconds to draw. Twenty seconds before each sampling time, 3 mL blood was drawn to clear the dead space in the catheter. The actual sample was drawn into a second syringe, and blood removal was completed at the precise times indicated. After sampling, the 3 mL of blood was reinfused and the catheter was flushed with heparinized saline. Arterial, portal vein, hepatic vein, and renal vein samples were drawn at 40, 50, and 60 minutes for determination of steady-state parameters. Arterial and portal vein samples were drawn simultaneously in the same manner as described earlier. Hepatic vein and renal vein samples were delayed by approximately 1 and 2 minutes, respectively. The delay in sampling should have had little effect, since glucagon concentration and other parameters were in steady state. The glucagon infusion rate was only changed after completion of all sampling at the 0-, 60-, 120-, and 180-minute time points.

Assays

Plasma glucose concentrations were determined using the glucose oxidase method with a Beckman glucose analyzer (Beckman Industries, Fullerton, CA). The intraassay coefficient of variation (CV) of determinations was 2%. Plasma insulin concentrations were determined using the double-antibody radioimmunoassay[33] with insulin tracer and antibodies provided by Linco (St Louis, MO). The interassay CV was 11% for insulin concentrations less than 200 pmol/L.

Plasma levels of immunoreactive glucagon (IRG) were measured in samples treated with 500 KIU/mL of a kallikrein-trypsin peptidase inhibitor, Trasylol (FBA Pharmaceuticals, New York, NY) using the radioimmunoassay technique outlined by Aguilar-Parada et al[34] and the 30-K antiserum of Unger (UT-Southwestern, Dallas, TX). The interassay CV was 20% at glucagon concentrations of 50 ng/L, 15% at 100 ng/L, and 10% at 300 ng/L. The 30-K antiserum cross-reacts with large- and small-molecular-weight proteins that lack glucagon's biologic activity and are not responsive to stimulation by arginine or to suppression by somatostatin.[35,36] Therefore, it was important to quantify the plasma concentration of the biologically active 3,500-d component of total glucagon immunoreactivity. Arterial samples from the beginning (0 minutes, middle (120 minutes), and end (240 minutes) of each experiment were obtained for filtering over 1 × 50-cm Biogel P-30 columns (Bio-Rad Labs, Richmond, CA) as previously described.[1] This procedure separated the samples into fractions containing peptides of differing molecular weights, so that plasma concentrations of ^{3500}IRG and other cross-reacting portions of total IRG could be determined. All the analysis of glucagon clearance and FE was performed using strictly the data for 3,500-d glucagon (^{3500}IRG = total IRG − average cross-reacting IRG). In some studies, plasma from portal vein, hepatic vein, and renal vein samples was subjected to separation over Biogel P-30 columns. The

amount of cross-reacting material in these samples was not statistically different from that found in arterial samples taken at the same time (data not shown).

Calculations

Arteriovenous-difference determinations. The FE and clearance rate (CR) of glucagon by the liver and kidney were determined using arteriovenous-difference techniques. The following formulas are derived using the Fick principle:

$$FE_{kidney} = \frac{^{3500}IRG_{ra} - ^{3500}IRG_{rv}}{^{3500}IRG_{ra}}, \quad \text{Eq 1}$$

$$FE_{liver} = \frac{(^{3500}IRG_{pv} \cdot F_{pv} + ^{3500}IRG_{ha} \cdot F_{ha}) - ^{3500}IRG_{hv} \cdot F_{hv}}{(^{3500}IRG_{pv} \cdot F_{pv} + ^{3500}IRG_{ha} \cdot F_{ha})}, \quad \text{Eq 2}$$

$$CR_{kidney} = FE_{kidney} \cdot F_{ra}, \quad \text{Eq 3}$$

$$\text{and } CR_{liver} = FE_{liver} \cdot F_{hv}, \quad \text{Eq 4}$$

where ra is the renal artery, rv is the renal vein, pv is the portal vein, ha is the hepatic artery, hv is the hepatic vein, and F is plasma flow. Renal plasma flow was determined using the *p*-aminohippurate balance method previously described.[32] Hepatic plasma flow was measured using both Doppler flow probes and the indocyanine green balance method previously described.[30,31] The two methods clearly show that hepatic plasma flow remained stable during each experiment. Doppler measurements were used for calculations because they quantify the individual contributions of the hepatic artery and portal vein to net hepatic plasma flow. The plasma flow as determined by dye-dilution techniques was less than that measured by Doppler flow probes. Using the flows from dye dilution would have resulted in somewhat smaller values for net hepatic glucagon clearance without substantially changing the conclusions of the report. FE and clearance were calculated for each individual dog at each individual steady-state measurement time point. The reported values are averages of these individual determinations and not values obtained from average hormone concentrations and average flows.

Hepatic glucose production. During each period of steady-state glucagon concentrations, we also estimated hepatic glucose production by calculating net hepatic glucose balance (NHGB):

$$NHGB = 0.73 \cdot [(PG_{pv} \cdot F_{pv} + PG_{ha} \cdot F_{ha}) - PG_{hv} \cdot F_{hv}], \quad \text{Eq 5}$$

where PG is the plasma glucose concentration, F is blood flow, and 0.73 is an experimentally determined factor used to convert plasma glucose to whole-blood glucose concentrations.[29]

Mathematical Modeling

The results of these experiments were modeled using compartmental analysis. Parameters of the compartmental model were estimated for each individual experiment using nonlinear least-squares algorithms as implemented in CONSAM.[12] The parameters of individual experiments were then averaged to obtain overall estimates for parameters of glucagon kinetics. In identifying the kinetic parameters, the error in the measurement of glucagon concentrations was assumed to be additive and uncorrelated, with an experimentally determined standard deviation that decreases as the glucagon concentration increases (note the CVs of the glucagon assay listed earlier).

Statistics

Differences between measurements at different steady states were compared using a paired, two-tailed *t* test with $\alpha = .05$. Analysis was performed with the Systat statistical package on an IBM-compatible personal computer.

RESULTS

Glucose and Insulin Concentrations

Arterial plasma glucose concentrations (Fig 1) remained clamped at an average of 13.9 ± 0.1 mmol/L throughout the entire study. The CV of the glucose concentrations within a study ranged from 4% to 12%. Peripheral somatostatin infusion with portal insulin replacement at 2.4 pmol · kg^{-1} · min^{-1} maintained arterial plasma insulin concentrations at basal levels during the entire study. There was a small but statistically significant increase of insulin levels from 70 ± 4 to 95 ± 12 pmol/L by the end of the experiment. This was probably due to increased insulin secretion despite attempted somatostatin blockade of the endocrine pancreas, since elevated plasma glucose and glucagon levels can both stimulate insulin secretion.

Glucagon Concentrations

The total plasma glucagon immunoreactivity was determined for every sample. Arterial samples taken at 0, 120, and 240 minutes were passed over gel-filtration columns to separate the physiologically active 3,500-d glucagon (^{3500}IRG) from cross-reacting material. The average amount of cross-reacting material in arterial plasma samples was 76

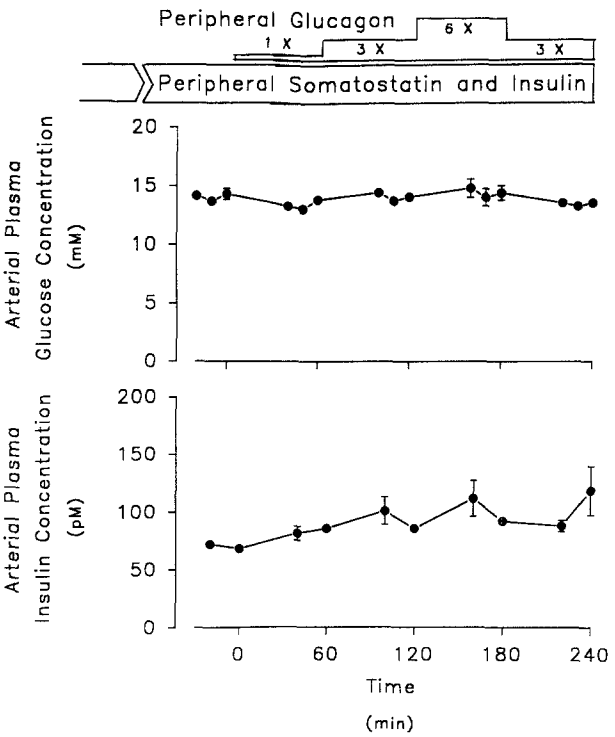

Fig 1. Arterial plasma glucose and insulin concentrations during a 4.5-hour glucose clamp. Peripheral somatostatin (0.8 μg · kg^{-1} · min^{-1}) and intraportal insulin (2.4 pmol · kg^{-1} · min^{-1}) were infused. Peripheral glucagon was infused at 1.0 ng · kg^{-1} · min^{-1} from 0 to 60 minutes, 3.0 from 60 to 120 minutes, 6.0 from 120 to 180 minutes, and 3.0 from 180 to 240 minutes.

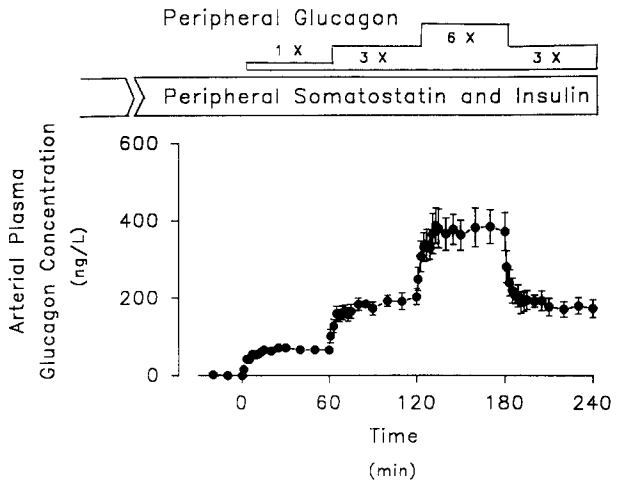

Fig 2. Arterial plasma concentrations of ^{3500}IRG during a 4.5-hour glucose clamp. Conditions as in Fig 1.

ng/L (range, 65 to 92), and the level of this material remained constant over 4 hours in each individual animal.

Samples from the control period, when no glucagon was infused, contained only cross-reacting material and no ^{3500}IRG, and the glucagon levels in the femoral artery, hepatic vein, portal vein, and renal vein were not statistically different. Therefore, the level of total plasma immunoreactivity measured in the control period was assumed to equal the level of cross-reacting material in every glucagon sample. This value was subtracted from the measured levels of glucagon immunoreactivity to determine the levels of ^{3500}IRG. Arterial plasma concentrations of 3,500-d IRG are shown in Fig 2. The average ^{3500}IRG was 0 ng/L during the control period, 68 ± 6, from 40 to 60 minutes, 195 ± 19 from 100 to 120 minutes, 378 ± 47 from 160 to 180 minutes, and 181 ± 20 from 220 to 240 minutes.

Hepatic and Renal Clearance of Glucagon

Average arterial, hepatic vein, portal vein, and renal vein plasma glucagon concentrations for each glucagon infusion step are listed in Tables 1 and 2. Hepatic artery, portal vein, and renal blood flow are also listed in the two tables. The hepatic vein catheter did not function properly in dog no. 1, and the renal vein catheter did not function in dog no. 2. Therefore, values listed in each table are averages for only four of the five dogs studied. Arterial plasma glucagon concentrations changed in a linear manner as the peripheral glucagon infusion rate was changed. Hormone concentrations in the other veins also changed linearly. Both hepatic and renal blood flow remained constant as the peripheral glucagon infusion was altered. The FE of glucagon by the liver and kidneys was 17.9% ± 4.1% and 28.6% ± 6.3%, respectively (P = NS between glucagon infusion steps). The clearance of glucagon by the liver and kidneys was 4.2 ± 0.8 and 2.9 ± 0.6 mL · kg^{-1} · min^{-1}, respectively (P = NS between glucagon infusion steps).

Compartmental Model

Arterial plasma ^{3500}IRG for each study was first fit to an equation with one exponential or two exponentials as described earlier. Not all experiments could be fit by an equation with the sum of two exponentials, and if the nonlinear identification algorithm converged, standard deviations of the parameter estimates were unacceptably high. Therefore, glucagon kinetics were best described using a compartmental model with a single pool that was the site of entry for the infused glucagon and the site of irreversible glucagon removal.

Parameter estimates of the glucagon kinetics model included the distribution volume (V_d) and the fractional clearance rate (FCR) of glucagon. Parameter estimates are listed in Table 3. The fit of the model to the experimental data of one example study is shown in Fig 3. Since the V_d of glucagon was not significantly different from the average plasma volume for dogs of 50 mL/kg, the data were again fit using a glucagon kinetic model with the V_d fixed at 50 mL/kg. The parameter estimates of this model are also listed in Table 3.

Hepatic Glucose Production

Hepatic glucose production was estimated by calculating the NHGB. Basal glucose and insulin concentrations were maintained throughout the experiment to minimize their effects on glucose production, so the increment in NHGB with each increment in glucagon should roughly reflect changes in glucagon stimulation of hepatic glucose production. NHGB was −19.4 ± 3.3 μmol · kg^{-1} · min^{-1} during the control period. Net uptake of glucose was expected under these conditions where glucose is elevated to two times basal, glucagon is absent, and insulin is at normal basal concentrations. As the glucagon infusion was incremented,

Table 1. Plasma Glucagon Concentrations in the Hepatic Artery, Portal Vein, and Hepatic Vein, and Hepatic Glucagon Clearance During Peripheral Glucagon Infusion at Three Different Rates in Dogs No. 2, 3, 4, and 5

	Period 1 (1 ng · kg^{-1} · min^{-1})	Period 2 (3 ng · kg^{-1} · min^{-1})	Period 3 (6 ng · kg^{-1} · min^{-1})	Period 4 (3 ng · kg^{-1} · min^{-1})
Hepatic artery ^{3500}IRG (ng/L)	63 ± 6	175 ± 5	326 ± 7	160 ± 7
Hepatic artery plasma flow (mL · kg^{-1} · min^{-1})	5.7 ± 0.8	5.8 ± 0.9	5.2 ± 1.2	5.9 ± 0.8
Portal vein ^{3500}IRG (ng/L)	64 ± 6	159 ± 12	298 ± 17	152 ± 11
Portal vein plasma flow (mL · kg^{-1} · min^{-1})	17.9 ± 2.4	19.6 ± 2.3	19.4 ± 2.5	18.9 ± 2.1
Hepatic vein ^{3500}IRG (ng/L)	55 ± 8	145 ± 14	249 ± 19	122 ± 10
FE_{liver} (%)	17.3 ± 6.8	12.9 ± 5.0	19.8 ± 4.7	21.5 ± 5.2
Hepatic clearance (mL · kg^{-1} · min^{-1})	4.0 ± 1.4	2.9 ± 1.0	4.8 ± 1.0	5.1 ± 1.1

NOTE. Values are the mean ± SEM.

Table 2. Plasma Glucagon Concentrations in the Renal Artery and Renal Vein and Renal Glucagon Clearance During Peripheral Glucagon Infusion at Three Different Rates in Dogs No. 1, 3, 4, and 5

	Period 1 (1 ng · kg^{-1} · min^{-1})	Period 2 (3 ng · kg^{-1} · min^{-1})	Period 3 (6 ng · kg^{-1} · min^{-1})	Period 4 (3 ng · kg^{-1} · min^{-1})
Renal artery ^{3500}IRG (ng/L)	70 ± 7	198 ± 23	389 ± 73	183 ± 30
Renal vein ^{3500}IRG (ng/L)	51 ± 8	126 ± 7	283 ± 29	134 ± 10
Renal plasma flow (mL · kg^{-1} · min^{-1})	10.1 ± 0.8	10.1 ± 0.8	10.7 ± 0.8	11.2 ± 0.6
FE_{kidney} (%)	27.9 ± 7.9	33.8 ± 5.9	24.8 ± 5.7	27.8 ± 7.3
Renal clearance (mL · kg^{-1} · min^{-1})	2.6 ± 0.7	3.2 ± 0.4	2.5 ± 0.5	3.0 ± 0.8

NOTE. Values are the mean ± SEM.

NHGB was elevated to −11.7 ± 3.9 μmol · kg^{-1} · min^{-1} from 40 to 60 minutes, 11.1 ± 5.6 from 100 to 120 minutes, 26.7 ± 4.4 from 160 to 180 minutes, and 2.8 ± 3.3 from 220 to 240 minutes. The positive values during the final three steady-state periods reflect the transition of liver from net uptake to net production in response to stimulation by glucagon. Figure 4 shows a plot of NHGB versus ^{3500}IRG concentration for each of the steady-state periods.

DISCUSSION

The aim of this study was to examine glucagon metabolism and distribution using both the compartmental-modeling approach and steady-state organ-balance techniques. To that end, experiments were conducted in conscious dogs 2 weeks following surgical placement of catheters and Doppler flow probes. Arterial plasma glucose and insulin concentrations were clamped, so that these factors would not vary and alter glucagon kinetics. We were particularly concerned that elevated insulin would compete with any nonspecific proteases involved in glucagon clearance, so insulin was infused at basal concentrations and glucose was infused to maintain hyperglycemia at approximately 14 mmol/L at all concentrations of glucagon. Both compartmental-modeling and organ-balance techniques were used for simultaneous quantitation of glucagon kinetics in the whole body and in the liver and kidneys.

The use of compartmental modeling and rapid sampling of the transitions between steady states provided important information regarding the V_d and FCR of glucagon. The modeling analysis of plasma concentrations showed that glucagon has extremely rapid kinetics in the conscious dog. It was distributed in only one compartment that has approximately the same volume as plasma. Roughly one third to one fourth of glucagon was cleared from this compartment every minute. The whole-body metabolic clearance rate of glucagon calculated from the model averaged 17.6 ± 1.6 mL · kg^{-1} · min^{-1}. Steady-state measurements and noncompartmental analysis were used to provide a separate measure of whole-body glucagon clearance and to determine the hepatic and renal glucagon clearance. With this technique, the whole-body glucagon clearance rate averaged 17.2 ± 1.3 mL · kg^{-1} · min^{-1} and the hepatic and renal clearances of glucagon were 4.2 ± 0.8 and 2.9 ± 0.6 mL · kg^{-1} · min^{-1}, respectively. The plasma clearance rate of the hormone was the same at all concentrations evaluated in these experiments, demonstrating that glucagon kinetics are linear over the physiologic range of glucagon concentrations. Hepatic and renal glucagon clearance rates were also equivalent for all glucagon concentrations measured, indicating that the individual-organ hormone clearances are linear.

It is important that the present set of experiments were performed with conscious animals 2 weeks after surgical placement of indwelling catheters, as opposed to previous experiments conducted with anesthetized animals immediately following surgery.[1-7] General anesthesia is known to produce profound physiologic changes in the splanchnic vascular bed, liver, and kidney.[8] General anesthesia initiated by pentobarbital and maintained by halothane decreases intestinal blood flow by 59%, which would have

Table 3. Parameter Estimates for the Single-Compartment Model of Glucagon Kinetics With No Constraints for the V_d of Glucagon or With the V_d Fixed at Plasma Volume Estimated at 50 mL/kg

	V_d Unconstrained		V_d Constrained		
Study No.	V_d (mL/kg)	FCR (min^{-1})	V_d (mL/kg)	FCR (min^{-1})	PCR (mL · kg^{-1} · min^{-1})
1	48.8 (9)	0.23 (9)	50	0.22	11.2
2	65.7 (15)	0.28 (15)	50	0.37	18.4
3	44.6 (26)	0.44 (25)	50	0.39	19.6
4	27.5 (15)	0.66 (15)	50	0.36	18.2
5	111.2 (11)	0.18 (10)	50	0.40	20.0
Average*	59.9 ± 14.3	0.36 ± 0.10	50	0.35 ± 0.03	17.6 ± 1.6

NOTE. Values in parentheses are CVs of parameter estimates.
*Mean ± SEM.

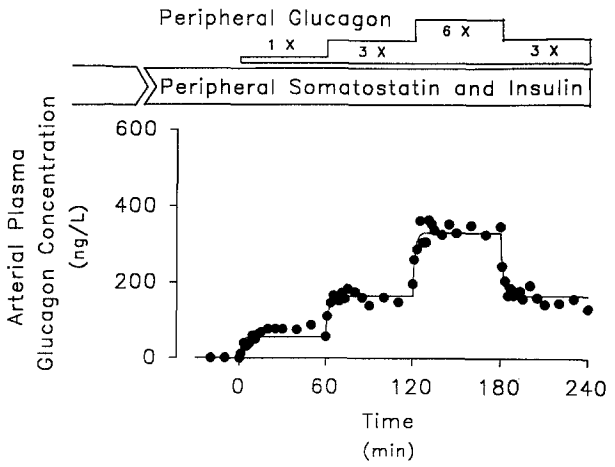

Fig 3. Model fit of ^{3500}IRG concentrations measured in dog no. 4 during peripheral glucagon infusion as in Fig 1.

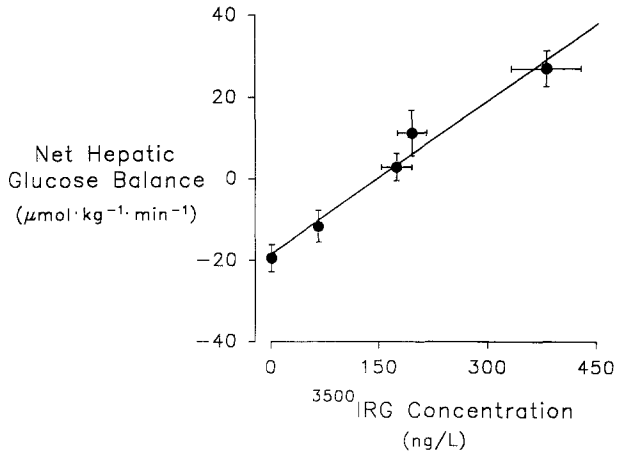

Fig 4. Plot showing NHGB *v* ^{3500}IRG concentration during peripheral glucagon infusion as in Fig 1. Glucose concentrations are clamped at approximately 2 times basal, and insulin concentrations are clamped at basal levels. The slope of the line was 0.127 μmol · kg^{-1} · min^{-1} of glucose per ng/L glucagon. The regression coefficient was 0.98.

profound effects on portal vein blood flow to the liver.[37] It is well known that barbiturate anesthesia also induces changes in hepatic enzymes, particularly the cytochrome P-450 system important for oxygenation and clearance of many drugs and toxins. For the kidneys, all measures of renal function including renal blood flow, glomerular filtration rate, urinary flow rate, and electrolyte excretion are altered during general anesthesia.[8] All these effects of general anesthesia occur independently of catecholamine changes associated with the stress resulting from surgical manipulations, so even carefully monitored experiments that minimize the stress to anesthetized animals could have results confounded by effects of anesthesia.

Despite the effects of anesthesia, glucagon clearance in previous studies conducted in anesthetized dogs following surgical placement of catheters was comparable to that observed in the present set of experiments. Polonsky et al[2,3] noted that the whole-body metabolic clearance rate of glucagon was 16.5 ± 0.8 mL · kg^{-1} · min^{-1} in anesthetized dogs. In addition, they determined that the hepatic and renal clearance rates of glucagon were 4.2 ± 0.3 and 4.7 ± 0.5 mL · kg^{-1} · min^{-1}, respectively. Hepatic glucagon clearance was identical in conscious and anesthetized animals, whereas renal clearance in the present studies was less than that observed previously. The difference between the studies was explained by diminished renal FE in our experiments (28.6% ± 6.3% *v* 43.7% ± 3.9%), because the renal plasma flows were equal. The renal FE in the present study is comparable to the FE of 23% Emmanouel et al[4] observed in normal rats and the 25% Lefebvre et al[6] observed in anesthetized dogs. Overall, the parameters of glucagon metabolism in the conscious dog resemble the values determined in anesthetized animal models, so the stresses of surgery and anesthesia seem to have minimal effects on glucagon clearance.

Since clearance by the liver and kidneys can only account for approximately 40% of glucagon degradation, other sites must be responsible for a significant portion of the irreversible removal of glucagon. The gastrointestinal tract would seem to be a likely candidate. It receives a significant percentage of the cardiac output and is composed of several tissues that are metabolically very active. The present data do not support the possibility of an important role for the gastrointestinal tract in metabolizing glucagon, since there is only a small and statistically insignificant arteriovenous difference of glucagon across the gut. Preliminary studies reported by Jaspan et al[1] suggested that there is some gut clearance of glucagon with a FE of approximately 10%, but subsequent studies by the same researchers did not highlight a significant role for the gut in glucagon clearance.[2,3] Erythrocytes are another possible site for glucagon degradation. Hildebrandt et al[38] reported that 20% of a 1-nmol/L glucagon load was degraded during a 4-hour incubation with intact human erythrocytes (packed cell volume, 45%). Furthermore, they noted that approximately 10% of the initial glucagon was associated with the cells and that 40% of the cell-associated glucagon consisted of degradation fragments. A protease termed the insulin-degrading enzyme has been isolated from erythrocytes.[39,40] The major function of this enzyme appears to be degradation of oxidatively damaged hemoglobin,[39] but it has also been shown to actively proteolize many other small peptides, including glucagon and insulin.[40]

Linear compartmental models are most often identified using data from a tracer bolus or a constant tracer infusion. The major advantage of tracers is that they exhibit linear, time-invariant dynamics as long as the system remains in steady state. In addition, tracers can often be more accurately measured than the tracee compound. Unfortunately, there are no true, high–specific activity tracers available to examine glucagon kinetics. Iodinated glucagon tracers have been shown to have binding characteristics different from native glucagon.[41,42] Tritiated glucagon tracers behave more like native glucagon, but their specific activity is so low that they cannot be used in trace amounts and still be accurately measured. Therefore, we used step inputs of native glucagon to identify the compartmental models. This approach is valid if glucagon kinetics are linear, and the data from these experiments clearly demonstrate the linearity of glucagon kinetics over the physiologic range of hormone concentrations.

One difficulty of the compartmental analysis was that the kinetics of glucagon were so rapid that the assumption of instantaneous mixing of the infused hormone in the accessible compartment may not have been met. This would have resulted in the measurement of falsely elevated plasma glucagon concentrations before complete mixing, which would lead to an underestimation of the V_d of glucagon. Therefore, the modeling of glucagon kinetics was repeated with the V_d fixed to the plasma volume. In this analysis, the FCR of hormone from the assumed plasma compartment was no different from that when no assumptions were made, suggesting that any delay in mixing had no significant effect on the conclusions of our analysis.

The description of glucagon kinetics obtained in the present set of experiments can easily be compared with similar studies investigating insulin kinetics.[10-14] Based on

experiments measuring plasma insulin concentrations during bolus and continuous insulin infusion in human subjects, Insel et al[13] formed a three-compartment model of insulin kinetics. The model consisted of a central plasma compartment with a volume of 45 ± 3 mL/kg and two remote compartments with a total plasma equivalent volume of insulin of 157 mL/kg. The FCR of insulin in the human subjects was 12 mL · kg^{-1} · min^{-1}, and is comparable to the clearance rate of 18.3 ± 1.5 measured for dogs.[3] In their analysis, Insel et al[13] also predicted that insulin action would occur in a remote compartment in which insulin equilibrated more slowly than in the central vascular compartment. The remote compartment presumably represented the extracellular fluid surrounding cells and interacting with receptor sites on the cell membrane. The present data indicate that glucagon is distributed in a single, rapidly equilibrating compartment with approximately the same volume as plasma. One would expect a multicompartmental model as noted for insulin. However, it is notable that Insel et al[13] had difficulty identifying parameters of the three-compartment model for individual experiments and had to constrain parts of the model so they could compute individual parameter values. Glucagon clearance in our model was approximately 50% more rapid than the insulin clearance they noted, which would make detecting multiple compartments even more difficult. Therefore, these results do not rule out movement of glucagon into the interstitium, but suggest that glucagon in the interstitium readily interacts with receptors and is cleared so rapidly that net flux from the interstitium back into the intravascular compartment is negligible compared with the rapid flux of glucagon out of plasma.

In conclusion, it is important to emphasize that glucagon is particularly well suited for a role in the minute-to-minute regulation of glucose homeostasis. Glucagon rapidly equilibrates in the plasma compartment following changes in glucagon secretion, allowing for minimal delay between changes in secretion and cellular signaling. In addition, glucagon is a potent stimulator of hepatic glycogenolysis and an inhibitor of glycogen synthesis that attains its maximal effects in just 15 minutes.[43] The combination of rapid equilibration in plasma and rapid activation of hepatic glucose production makes glucagon well suited for its role as a primary acute determinant of glucose homeostasis.

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