Rates of Glucagon Activation and Deactivation of Hepatic Glucose Production in Conscious Dogs

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To determine the time course of glucagon activation and deactivation of hepatic glucose production (HGP), studies were conducted in 18-hour fasted, conscious dogs. Somatostatin was infused with insulin replaced intraportally at 1.8 pmol · kg^{-1} · min^{-1} and glucagon replaced peripherally at 1.0 ng · kg^{-1} · min^{-1}. After a 2-hour control period, glucagon infusion was either (1) increased fourfold for 4 hours (GGN 4X), (2) increased fourfold for 30 minutes and returned to a basal rate for 3.5 hours (GGN 4X/1X), or (3) fixed at the basal rate for 4 hours (GGN 1X). In the latter two protocols, glucose was infused peripherally to match glucose concentrations observed during GGN 4X. Glucose turnover was determined by deconvolution with the impulse response of the glucose system described by a two-compartment, time-varying model identified from high-performance liquid chromatography (HPLC)-purified [3-^{3}H]glucose tracer data. In GGN 4X, HGP was stimulated from 15.2 ± 0.9 μmol · kg^{-1} · min^{-1} to 52.7 ± 6.5 μmol · kg^{-1} · min^{-1} after just 15 minutes, but it decreased over the subsequent 3 hours to a rate 25% above basal. In GGN 4X/1X, the increase in HGP during the first 30 minutes equaled that observed in GGN 4X, but when glucagon infusion was returned to basal, HGP decreased in 15 minutes to rates equal to those observed in GGN 1X. The times for half-maximal activation and deactivation of glucagon action were equal (4.5 ± 1.0 and 4.0 ± 1.1 minutes, respectively). The very rapid and sensitive hepatic response to glucagon makes pancreatic glucagon release a key component of minute-to-minute glucose homeostasis.

PREVIOUS EXPERIMENTS have used isotope dilution methods to characterize the time course of the hepatic response to a physiologic increase in arterial plasma glucagon concentrations in animals[1-3] and humans.[4,5] Such experiments have demonstrated that increased glucagon results in a marked stimulation of hepatic glucose production (HGP) that wanes over time. An example of the evanescent effect of glucagon can be found in early experiments from our laboratory, in which somatostatin was infused in conscious dogs and basal insulin secretion was replaced using an intraportal insulin infusion. Intraportal glucagon infusion for 3 hours at four times the basal secretion rate resulted in persistent hyperglycemia (12.1 ± 0.8 mmol/L). Glucose production was estimated from the dilution of [3-^{3}H]glucose tracer using Steele's equations for non–steady-state glucose turnover.[6,7] Endogenous glucose production increased from 16.1 ± 0.6 μmol · kg^{-1} · min^{-1} to 46.7 ± 5.5 μmol · kg^{-1} · min^{-1} by 15 minutes, but the effect of hyperglucagonemia on glucose production declined 85% over the next 3 hours.[2] The evanescent effect of glucagon was due to changes in glycogenolysis and not gluconeogenesis. In a previous study, we showed that elevated glucagon increased gluconeogenic efficiency; however, without an increase in the peripheral supply of gluconeogenic precursors, it was unable to change the net gluconeogenic rate.[3]

The "evanescence" of glucagon stimulation of glycogenolysis was a consistent finding in both dogs[1,2] and humans[4,5] and confirmed the previous observation of Plas and Nunez,[8] who first noted a refractoriness to glucagon stimulation of glycogenolysis in cultured fetal rat hepatocytes. Subsequent studies in conscious dogs[1] and in perfused rat liver[9] examined the importance of hyperglycemia in attenuating the glucagon-induced stimulation of HGP. These studies revealed that hyperglycemia did not directly inhibit glucagon's ability to stimulate glucose production above baseline, but did contribute to a decline in the basal glucose production rate. Thus, 40% of the decrease in glucose production was due to a hyperglycemia-induced decline in basal hepatic glucose output. The remainder (60%) was due to hepatic desensitization to glucagon's effect on glycogenolysis.

In the previous in vivo studies, glucose production was estimated using infusion of impure [^{3}H]glucose tracers with non–steady-state glucose turnover calculated with Steele's equations. It is now recognized that the inadvertent infusion of tritiated non-glucose contaminants[10,11] led to a systematic underestimation of HGP. This error can be readily avoided by separating non-glucose contaminants from [^{3}H]glucose with high-performance liquid chromatography (HPLC) before infusion.[11] Recent theoretical studies demonstrate that the use of Steele's equations[7,12] will underestimate HGP when the plasma specific activity is decreasing rapidly.[10,13] Avoiding such non–steady-state errors requires additional experimental and/or mathematical effort.

Several different strategies can be used to provide improved estimates of non–steady-state glucose production,[10,14-17] but we have chosen to use a deconvolution method described previously.[14,15] The method is a two-stage approach that uses a variable infusion of HPLC-purified glucose tracer combined with frequent arterial blood sampling to identify a time-varying, two-compartment model[16] of glucose kinetics that accurately relates the measured tracer concentration and the known tracer input. Once a model of glucose kinetics is identified from tracer data, deconvolution can be used for estimating HGP from the measured plasma glucose concentration. The advantages of this approach are as follows: (1) it makes no prior assumption on the shape of the HGP response; (2) unlike the Steele model, it is not

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prone to underestimate non–steady-state glucose production; and (3) it is sensitive enough to examine the hepatic response to glucagon on a minute-to-minute basis. Therefore, the aim of the present set of experiments was to use a purified glucose tracer and more accurate modeling techniques to examine the time course of activation of HGP in response to a step increase in glucagon and the time course of the response decay during a step decline in glucagon.

MATERIALS AND METHODS

Animals and Surgical Procedures

Experiments were performed on mongrel dogs (18 to 28 kg) of either sex that had been fed once daily a standard diet of Kal Kan beef dinner (Vernon, CA) and Purina Dog Chow (St Louis, MO) (51% carbohydrate, 31% protein, 11% fat, and 7% fiber by dry weight). Dogs were housed in a surgical facility that meets American Association for Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by our University Animal Care Committee. Two weeks before each set of experiments, a laparotomy was performed with the animal under general anesthesia. Using standard sterile techniques previously described,[18] Silastic catheters (Dow Corning, Midland, MI; 0.03 in ID) were placed in the jejunal and splenic veins for infusion into the hepatic portal system. A Silastic catheter (0.04 in ID) was also inserted into the left femoral artery for blood sampling. The free ends of the catheters were placed in subcutaneous pockets, and the skin incisions were closed. Penicillin G (500 kU) was administered postoperatively, and ampicillin (1 g orally) was given with meals for 2 days.

Three days before each experiment, blood was withdrawn to determine the hematocrit and leukocyte count. Experiments were performed only on dogs with (1) a leukocyte count less than 18,000/μL, (2) a hematocrit greater than 35%, (3) a good appetite, and (4) normal stools. Dogs were fasted for 18 hours before each study. On the morning of an experiment, the catheter ends were exteriorized through incisions made under local anesthesia (2% lidocaine) and the catheters were flushed with heparinized saline (1 U/mL). Twenty-gauge Angiocaths (Becton Dickinson, Sandy, UT) were inserted into the cephalic and saphenous veins for infusion of hormones and tracers. Following the completion of a study, any dog that was to be used for a subsequent experiment was placed under general anesthesia. The catheters were filled with heparin (200 U/mL), cleaned with betadine, and placed in subcutaneous pockets. The animal was treated with antibiotics as before. Second or third experiments were performed after a 2-week delay, and then only if the dog met the health criteria already outlined.

Experimental Procedures

Each experiment consisted of a pancreatic clamp[19] period (mean, 100 minutes; range, 70 to 140), a 2-hour basal period, and a 4-hour experimental period. Each study began with peripheral somatostatin infusion at 0.8 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Glucagon was replaced peripherally at a basal rate of 1.0 $ng \cdot kg^{-1} \cdot min^{-1}$, which is 50% greater than the rate of 0.65 $ng \cdot kg^{-1} \cdot min^{-1}$ we use for portal glucagon replacement and is designed to match the basal glucagon concentration of the liver. Portal insulin infusion was started at 1.5 $pmol \cdot kg^{-1} \cdot min^{-1}$. Arterial plasma glucose concentrations were measured every 5 minutes, and the insulin infusion rate was adjusted to maintain glucose at basal levels. This continued until plasma glucose remained constant for 20 minutes without changing the insulin infusion rate. Following establishment of the pancreatic clamp, a primed-continuous infusion of HPLC-purified [3-^{3}H]glucose tracer was begun (≈2.0 μCi/kg bolus and ≈0.02 $\mu Ci \cdot kg^{-1} \cdot min^{-1}$ infusion). Arterial blood was sampled at frequent intervals to determine the plasma glucose concentration and radioactivity for modeling basal glucose kinetics. A second bolus of [3-^{3}H]glucose tracer was injected 2 hours into the experimental period to facilitate modeling of the change in glucose kinetics in response to hyperglucagonemia and hyperglycemia.

Paired protocols were performed in five animals. The first set of experiments, GGN 4X, determined the stimulation of HGP resulting from an increase in the peripheral infusion rate of glucagon to 4.0 $ng \cdot kg^{-1} \cdot min^{-1}$ for 4 hours. This infusion rate increases hepatic glucagon to levels often seen during hypoglycemia or exercise.[20,21] GGN 4X/1X determined the stimulation of HGP from an increase in the peripheral infusion rate of glucagon to 4.0 $ng \cdot kg^{-1} \cdot min^{-1}$ for 30 minutes followed by a return to infusion at 1.0 $ng \cdot kg^{-1} \cdot min^{-1}$. To control for the effects of glucose concentrations on glucose production, glucose was infused peripherally in GGN 4X/1X, to match arterial plasma glucose concentrations present in GGN 4×. A third set of experiments, GGN 1×, was conducted in two of five dogs used for the paired studies described and an additional dog not previously studied. The protocol provided a control for the effects of hyperglycemia per se on glucose production. Peripheral glucagon infusion remained fixed at 1.0 $ng \cdot kg^{-1} \cdot min^{-1}$ for 4 hours while glucose was infused to match arterial plasma glucose concentrations present in the previous two protocols. In all of the protocols, the insulin infusion remained fixed during the glucagon infusion periods.

In all three protocols, arterial blood samples were taken at frequent intervals for determination of plasma glucose concentration and radioactivity. Plasma glucagon concentrations were also determined at frequent intervals, and arterial insulin concentrations were measured every 30 minutes. During each study, approximately 20% of the total blood volume was removed and replaced with 2 vol saline.

Processing of blood samples. The collection and immediate processing of blood samples have been previously described.[22] Plasma glucose concentrations were determined using the glucose oxidase method[23,24] with a Beckman glucose analyzer (Beckman, Fullerton, CA). The coefficient of variation (CV) for this method was 2%. Plasma glucose radioactivity (dpm per milliliter) was determined by liquid scintillation counting of deproteinized samples that were dried to evaporate ^3H_2O and then reconstituted with 1 mL deionized water.[25] The CV for determinations was 5%.

Hormone assays. Plasma levels of immunoreactive insulin were measured using the double-antibody radioimmunoassay.[26] The interassay CV was 11% for basal insulin levels. Plasma levels of immunoreactive glucagon were measured in samples treated with 500 KIU/mL Trasylol using the radioimmunoassay technique outlined by Aguilar-Parada et al[27] and the 30-K glucagon antiserum. The interassay CV for glucagon was 15%.

Materials. Purified [3-^{3}H]glucose (11.5 mCi/mmol; New England Nuclear, Boston, MA) was used as the glucose tracer. Insulin was purchased from Squibb-Novo (Princeton, NJ), glucagon from Eli Lilly (Indianapolis, IN), and somatostatin from Bachem (Torrance, CA). Trasylol was obtained from FBA Pharmaceuticals (New York, NY). The tracer and antibodies for insulin assay were purchased from Linco (St Louis, MO), and the glucagon tracer was obtained from Novo Research Institute (Copenhagen, Denmark) and the 30-K glucagon antiserum from Dr R.H. Unger (Southwestern Medical Center, Dallas, TX).

Calculations and Models

Glucose production. Glucose production was estimated in a two-stage procedure that used both classic system identification methods and deconvolution.[14,15] For any linear, time-varying system, the input and output can be mathematically related by the convolution integral,

$$y(t) = \int_{-\infty}^{t} x(t,\tau)u(t)\,d\tau, \qquad \text{Eq 1}$$

where $y(t)$ is the system output, $x(t,\tau)$ is the impulse response of the system, and $u(t)$ is the system input. Whenever two of the three system components are known, the third component can be directly calculated.

The first stage of the glucose production determination was the well-known system identification problem. HPLC-purified [3-^{3}H]glucose was infused at a known rate, and plasma glucose radioactivity was measured. The unknown impulse response of the glucose tracer was described by a time-varying, two-compartment model identified from the plasma glucose radioactivity data. The model of glucose tracer kinetics is shown in Fig 1 and is described by the set of differential equations,

$$\dot{q}_1(t) = -\dot{k}_{01}(t) + k_{21})_{q1}(t) + k_{12q2}(t) + u(t) \quad \text{Eq 2}$$

$$\dot{q}_2(t) = -\dot{k}_{02} + k_{12})_{q2}(t) + k_{21q1}(t) \quad \text{Eq 3}$$

$$c(t) = q_1(t)/V_1 \quad \text{Eq 4}$$

$$z(t_s) = c(t_s) + e(t_s) \quad \text{Eq 5}$$

$$q_1(0) = q_2(0) = 0 \quad \text{Eq 6}$$

$$k_{01}(t) = \begin{cases} k_{01b} & -120 < t < 0 \\ k_{01f} + (k_{01b} - k_{01f})e - a01t & 0 < t < 240, \end{cases} \quad \text{Eq 7}$$

where $q_i(t)$ (dpm) is the mass of tracer in the icompartment; k_{ij} (per minute) values are fractional transfer rates of tracer from compartment j to compartment i; u(t) (dpm per minute) is the tracer input; c(t) (dpm per milliliter) is the tracer concentration in compartment 1; V_1 (milliliters) is the volume of compartment 1; and $z(t_s)$ is the discrete tracer concentration sampled at time t_s, which is equal to the actual value $c(t_s)$ with some measurement error $e(t_s)$. The measurement error of plasma glucose radioactivity was assumed to be additive, white, with a zero mean and an experimentally determined standard deviation.

The time-varying, two-compartmental model is based on the model originally constructed by Ferrannini et al,[16] in which glucose disposal from compartment 1 represents insulin-independent glucose uptake and glucose disposal from compartment 2 represents insulin-dependent glucose uptake. Insulin concentrations remain constant at basal levels in the present study, so k_{02}, k_{12}, and k_{21} were fixed while k_{01} was allowed to change in response to hyperglycemia. The time course of $k_{01}(t)$ was chosen by fitting data with multiple different equations for $k_{01}(t)$. The exponential equation provided the best fit of available data (results not shown). The model contained seven unknown parameters (V_1, k_{01b}, k_{01f}, a_{01}, k_{21}, k_{12}, and k_{02}) and was not uniquely identifiable without including an additional relationship. As suggested by Ferrannini et al,[16] k_{01b} was constrained so that glucose disposal from compartment 1 was equal to 75% of total basal glucose utilization ($R_{D\,basal}$). Thus, $q_1 \cdot k_{01b} = 0.75 \cdot R_{D\,basal}$, where R_{Dbasal} was determined using the steady-state relation, $R_{D\,basal}$ = [3-^{3}H]glucose tracer infusion rate steady-state basal [3-^{3}H]glucose specific activity. The selection of this model and analysis of its identifiability are described in greater detail elsewhere.[9] Model parameters were identified using the SAAM simulation and modeling software, which uses nonlinear least-squares estimation algorithms to fit models defined by differential equations.[28] Almost all parameters were estimated with acceptable precision, the residuals (differences between measured and calculated plasma glucose radioactivity) were within the limits of measurement error (CV, 5%), and there were no systematic deviations from the experimental data as determined by the runs test.

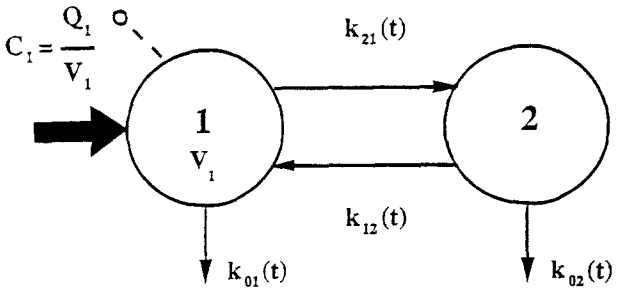

Fig 1. Diagram of general time-varying, 2-compartment model of glucose kinetics. Dotted line represents concentration measurement of accessible compartment, and large arrow represents input for this compartment. Compartment 1 includes insulin-independent tissue (central nervous system, plasma, etc), and compartment 2 includes insulin-dependent tissues (adipose tissue, muscle, etc).

The second stage of the glucose production determination used deconvolution to calculate the unknown production from the measured plasma glucose concentration and the previously identified model of glucose kinetics. Because deconvolution by simple matrix inversion and multiplication was overly sensitive to measurement error, we used an algorithm proposed by Twomey[29] and Phillips[30] described in previous publications.[14,15] Two protocols in the present study required infusion of exogenous glucose, so additional steps were needed in estimating HGP. The exogenous infusion was incorporated by estimating the glucose concentration resulting from the infusate with model simulation, subtracting this component from the plasma glucose measurements to yield glucose concentrations resulting from endogenous production, and only then performing the deconvolution. The computer program for the deconvolution was written in FORTRAN and executed on an IBM-compatible computer.

Statistical Analysis

Statistical analysis was performed using paired *t* tests between the GGN 4X and GGN 4X/1X protocols and unpaired analysis for other comparisons. Because comparison of two groups point by point with *t* tests over a large number of repeated time-dependent samples would yield many false-positives due to type 1 error, specific parameters of the response, ie, total area under the curve or rate of increase, etc., were compared. All comparisons were made with the probability of type 1 error set to 5% or less and had the statistical power to detect differences on the order of 15% to 20%, depending on the parameter of interest.

RESULTS

Hormone Levels

Arterial plasma glucagon concentrations were significantly different between the three experimental groups (Fig 2). Peripheral glucagon infusion at 1.0 ng · kg^{-1} · min^{-1} maintained basal arterial plasma glucagon levels of 78 ± 3, 86 ± 7, and 85 ± 4 ng/L in GGN 4X, GGN 4X/1X, and GGN 1X, respectively. In GGN 4X and GGN 4X/1X, when peripheral glucagon infusion was increased to 4.0 ng · kg^{-1} · min^{-1}, the glucagon concentration increased to 210 ± 13 and 218 ± 11 ng/L, respectively ($P < .01$ *v* baseline). Glucagon concentrations remained elevated for 4 hours in GGN 4X, but decreased to basal levels in GGN 4X/1X when the glucagon infusion was returned to 1.0 ng · kg^{-1} · min^{-1}. The times for the glucagon level to increase and decrease were similar. In GGN 1X, glucagon was maintained at basal levels throughout the study. Arterial plasma insulin concentrations were stable and similar in each experimental protocol (69 ± 8, 62 ± 4, and 55 ± 7 pmol/L in the three protocols, respectively; Fig 2).

Plasma Glucose and Exogenous Glucose Infusion Rates

Arterial plasma glucose concentrations were similar in the three experimental groups (Fig 2). Basal plasma glucose levels

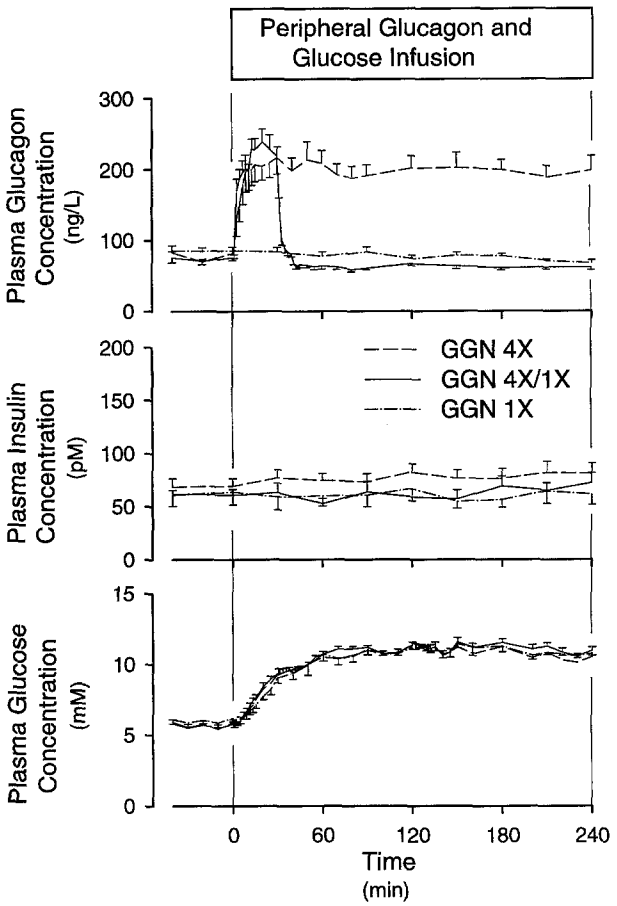

Fig 2. Arterial plasma glucagon, insulin, and glucose levels during 4 hours of hyperglycemia induced by peripheral glucagon and glucose infusions. The GGN 4X group (n = 5) received peripheral glucagon at 4 ng · kg^{-1} · min^{-1} for 4 hours. The GGN 4X/1X group (n = 5) received 4 ng · kg^{-1} · min^{-1} glucagon for 30 minutes and then basal glucagon (1 ng · kg^{-1} · min^{-1}) for 3.5 hours. The GGN 1X group (n = 3) received basal glucagon for 4 hours. The latter 2 groups were infused with glucose to match the levels noted in GGN 4X. Values are the mean ± SE.

were 5.7 ± 0.1, 5.7 ± 0.3, and 6.1 ± 0.3 mmol/L in GGN 4X, GGN 4X/1X, and GGN 1X, respectively. The glucose level doubled ($P < .01$ *v* baseline) in each group during the experimental period, reaching steady-state levels of 11.4 ± 0.1, 11.3 ± 0.3, and 11.2 ± 0.1 mmol/L. In GGN 4X, this increase was solely due to glucagon stimulation of HGP. In the other protocols, glucose was infused peripherally so that the glucose concentrations noted during hyperglucagonemia were matched.

Glucose Turnover

Glucose kinetics were modeled using a time-varying, two-compartment model that was identified from the [3-^{3}H]glucose tracer data. Table 1 lists the parameter estimates of the model along with their precision expressed as the CV. The mean of the residuals was zero, and there were no systematic deviations from zero detected using the runs test for goodness of fit. Almost every parameter was estimated with an acceptable degree of precision, often with a CV less than 10%. In two experiments of the GGN 1X protocol, the time constant was difficult to define with precision because of small differences between k_{01b} and k_{01f}, but the resulting models provided a good fit of experimental data. Figures showing the model fit of [3-^{3}H]glucose tracer data using the time-varying, two-compartment model were published previously.[15]

Glucose utilization was directly determined from the time-varying, two-compartment model. Figure 3 shows mean results for the total glucose utilization and the utilization from compartments 1 and 2 for these studies. All three of these measures were similar between the experimental groups. Basal glucose utilization was 15.1 ± 0.8, 13.2 ± 0.6, and 14.2 ± 0.5 μmol · kg^{-1} · min^{-1} in GGN 4X, GGN 4X/1X, and GGN 1X, respectively, and increased to 19.7 ± 3.6, 21.9 ± 1.1, and 23.8 ± 2.8 μmol · kg^{-1} · min^{-1} ($P < .01$ *v* baseline) with hyperglucagonemia and hyperglycemia. Although there was a trend toward a smaller increase in glucose utilization in the GGN 4X group, this did not reach statistical significance at *P* less than .05.

The portion of the glucose concentration originating from exogenous infusion was derived by integrating the known glucose infusion rate and the impulse response of the glucose system (determined from the tracer model). The exogenous glucose infusion rates for the GGN 1X and GGN 4X/1X protocols are shown in Fig 4 along with the derived glucose concentrations. These values were subtracted from the actual measured glucose concentrations to determine the portion of glucose arising from endogenous production that is shown in Fig 5 along with the tracer-determined HGP derived by deconvolution. Hyperglycemia induced by glucose infusion in the presence of basal glucagon and insulin (GGN 1X) suppressed HGP to 75% below the basal rate (14.3 ± 3.7 μmol · kg^{-1} · min^{-1} to 3.4 ± 1.7 μmol · kg^{-1} · min^{-1}, $P < .01$ *v* baseline). When the glucagon infusion was increased fourfold, there was a rapid stimulation of production from 15.2 ± 0.9 to a peak of 52.7 ± 6.5 μmol · kg^{-1} · min^{-1} within 15 minutes ($P < .01$ *v* GGN 1X). This stimulation exhibited a spike-decline pattern, with HGP decreasing over the subsequent 3 hours to 17.2 ± 1.7 μmol · kg^{-1} · min^{-1}, a rate still much higher than observed in the basal glucagon control group ($P < .01$ *v* GGN 1X). In GGN 4X/1X, HGP increased over the first 30 minutes when glucagon was infused at 4.0 ng · kg^{-1} · min^{-1} ($P < .01$ *v* GGN 1X) and was equal to the increase noted in GGN 4X. When the glucagon infusion was returned to 1.0 ng · kg^{-1} · min^{-1}, HGP decreased in 15 minutes to rates equal to those observed in the basal glucagon control group, GGN 1X.

Activation and Deactivation of HGP

Any change in glucagon concentration was rapidly reflected by stimulation or inhibition of HGP. HGP was maximally stimulated 15 minutes following an increase in the glucagon infusion rate from 1.0 to 4.0 ng · kg^{-1} · min^{-1}. The time required to reach half-maximal stimulation was just 4.5 ± 1.0 minutes. HGP was also rapidly inhibited following a decrease in the glucagon infusion rate, decreasing in just 15 minutes to a rate not different from that seen in basal glucagon controls. The time required to reach half-maximal deactivation was just 4.0 ± 1.0 minutes (Fig 6).

DISCUSSION

The aim of the present experiments was to use a purified glucose tracer and more accurate techniques for measuring glucose production to examine the time course of activation of

Table 1. Parameter Estimates for the Time-Varying, Two-Compartment Model of Glucose Kinetics During 4 Hours of Hyperglycemia Induced by Peripheral Glucagon and Glucose Infusions

Dog No./Study	V_1 (mL/kg)	k_{02} (min^{-1})	k_{12} (min^{-1})	k_{21} (min^{-1})	k_{01b} (min^{-1})	k_{01} (min^{-1})	1/a (min)
1							
GGN 4X	137 (4)	0.0046	0.0809 (11)	0.0896 (12)	0.0167 (12)	0.0121 (15)	11 (87)
GGN 4X/1X	139 (2)	0.0053	0.0607 (12)	0.0444 (10)	0.0131 (4)	0.0110 (5)	10 (66)
GGN 1X	136 (4)	0.0044	0.0644 (15)	0.0664 (14)	0.0151 (7)	0.0086 (8)	10 (80)
2							
GGN 4X	141 (3)	0.0048	0.0636 (11)	0.0674 (13)	0.0173 (5)	0.0109 (10)	39 (34)
GGN 4X/1X	134 (3)	0.0032	0.0388 (15)	0.0531 (10)	0.0147 (8)	0.0099 (9)	46 (25)
3							
GGN 4X	133 (5)	0.0044	0.0696 (10)	0.0691 (10)	0.0145 (5)	0.0073 (6)	19 (37)
GGN 4X/1X	117 (11)	0.0032	0.0559 (9)	0.0822 (10)	0.0154 (11)	0.0135 (13)	1 (264)
4							
GGN 4X	144 (2)	0.0054	0.0791 (10)	0.0675 (12)	0.0157 (4)	0.0079 (5)	4 (99)
GGN 4X/1X	131 (5)	0.0059	0.0998 (15)	0.0837 (20)	0.0162 (7)	0.0077 (10)	38 (26)
5							
GGN 4X	150 (5)	0.0036	0.0678 (11)	0.0585 (12)	0.0102 (5)	0.0063 (8)	40 (43)
GGN 4X/1X	132 (4)	0.0034	0.0870 (15)	0.0795 (18)	0.0111 (8)	0.0108 (10)	1 (1210)
GGN 1X	141 (4)	0.0037	0.0850 (18)	0.0651 (20)	0.0114 (9)	0.0107 (6)	4 (787)
6							
GGN 1X	153 (5)	0.0040	0.0673 (16)	0.0667 (19)	0.0135 (9)	0.0136 (7)	1 (621)

NOTE. Values in parentheses are the CV.

HGP in response to a step increase in glucagon, as well as the time course of the response decay when a step decrease in glucagon was achieved. The present experiments are the first to estimate the time for activation and deactivation of HGP by glucagon in an in vivo model. A fourfold increase in glucagon infusion in the presence of constant, basal insulin resulted in an increase of HGP from a basal rate of 15.2 ± 0.9 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ to a peak of 52.7 ± 6.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ in 15 minutes. The time to reach half-maximal stimulation of HGP was rapid, just 4.5 ± 1.0 minutes. When glucagon infusion was returned to a basal rate after being elevated for 30 minutes, glucose production decreased in just 15 minutes to rates not different from those seen in the hyperglycemic control protocol. The time to reach half-maximal deactivation of HGP was 4.0 ± 1.1 minutes. It must be remembered that the changes in glucagon input were square wave, thus speeding the response relative to that seen when secretion itself has to turn on and off.

These results clearly demonstrate that the time constant for activation of HGP in response to glucagon is approximately equal to the time constant for the response decay. This contradicts the findings of Weigle et al[31,32] in perifused hepatocytes that suggested the decay time constant is slower than the activation time constant. One explanation for the disparity between the in vivo determinations and perifused-hepatocyte data could be the difference in delivery of glucagon to the cells. In nonrecirculating perifusion systems, the kinetics for distributing hormone to the hepatocytes and removing it from the cells were very rapid ($t_{1/2} \approx 1$ minute) and the increase and the decrease in glucagon concentration were not symmetrical: the decrease was slower. In our conscious dog study, the hormone was distributed to and removed from hepatocytes more slowly ($t_{1/2}$, 2.4 minutes[33,34]) and the increase and the decrease in hormone concentrations were symmetrical. A second explanation for the disparate results could be that the perifusion medium did not contain other hormones (insulin and catecholamines) and substrates (amino acids and fatty acids) that have been shown to alter the hepatocyte response to glucagon.[35,36] Weigle et al[31,32] postulated that the delayed recovery time for glucagon action, as noted in their perifused-hepatocyte system, would cause pulsatile hormone administration[37] to have a greater effect than an equal amount of hormone given at a constant rate. Equivalent response times, as seen in the present in vivo system, would explain why, on the contrary, pulsatile glucagon administration has not been shown to have significantly greater effects than constant glucagon with in vivo models.[33,38,39]

Increased glucagon results in a marked stimulation of HGP that wanes over time. The initial stimulation of HGP was 350% above the basal production rate, but it decreased to only 15% above basal after 3 hours, suggesting significant hepatic desensitization to glucagon. Plas and Nunez[8] long ago recognized that prior exposure to very high glucagon concentrations desensitizes hepatocytes to subsequent hormone exposure. Similarly, physiological hyperglucagonemia initially increased HGP in dogs[1,2] and men,[4,5] but the effect dissipated with prolonged exposure to the hormone. Hepatic desensitization to glucagon action has been associated with a decrease in the formation of cyclic adenosine monophosphate (cAMP).[40,41] Apparently, multiple molecular pathways are responsible for modulating glucagon's effect on intracellular cAMP. Glucagon binding uncouples the glucagon receptors from their regulatory guanosine triphosphate–binding proteins (G_s).[42] This uncoupling of receptor-G_s complexes transforms the receptors to a lower-affinity binding state and removes their ability to stimulate adenylate cyclase. This is a short-term negative-feedback mechanism, since receptor-G_s complexes are rapidly recoupled when glucagon is removed. A long-term feedback mechanism becomes apparent with more prolonged hormonal exposure. The low-affinity receptors are internalized and the number of glucagon binding sites is reduced.[6,42-44] When glucagon stimulation is removed, it takes several hours for the receptors to return to the cell membrane and restore the normal sensitivity to glucagon. Another pathway has been implicated as a form of short-term negative feedback. The cAMP-dependent protein kinase phosphorylates and activates low-K_m phosphodiesterases that in-

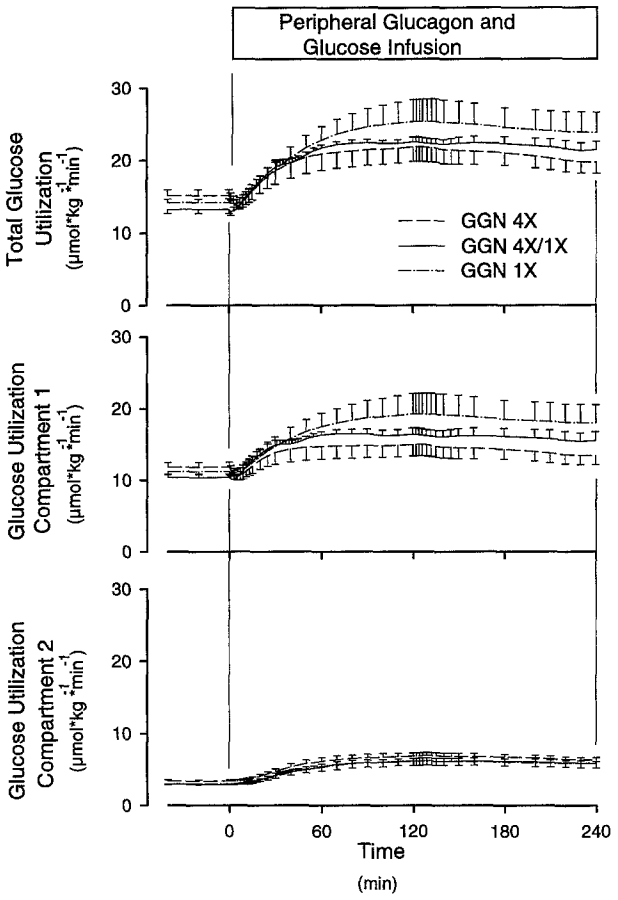

Fig 3. Glucose utilization determined from the time-varying, 2-compartment model of glucose kinetics during 4 hours of hyperglycemia induced by peripheral glucagon and glucose infusions. The GGN 4X group (n = 5) received peripheral glucagon at 4 ng · kg^{-1} · min^{-1} for 4 hours. The GGN 4X/1X group (n = 5) received 4 ng · kg^{-1} · min^{-1} glucagon for 30 minutes and then basal glucagon (1 ng · kg^{-1} · min^{-1}) for 3.5 hours. The GGN 1X group (n = 3) received basal glucagon for 4 hours. The latter 2 groups were infused with glucose to match the levels noted in GGN 4X. Values are the mean ± SE.

crease the clearance of cAMP within the cell.[45,46] Progressive activation of these phosphodiesterases could also contribute to a time-dependent decrease in glucagon action. It is also possible that depletion of hepatic glycogen could cause desensitization to glucagon action. Eighteen-hour fasted dogs have about 70 g hepatic glycogen, which would have decreased 20% to 25% over 4 hours of hyperglucagonemia. The decrease in HGP was much greater (~70%) than the decrease in hepatic glycogen, suggesting that it resulted from something other than or in addition to glycogen depletion.

The magnitude of the decrease in glucose production when glucagon infusion was returned to basal after 30 minutes of hyperglucagonemia revealed important information about the mechanism responsible for desensitization of the liver to glucagon action. HGP decreased to the rate observed in the hyperglycemic control protocol, but did not go below this "baseline" glucose production. This suggests that the mechanism responsible for inhibiting glucagon action over 4 hours of hyperglucagonemia may not be significantly activated after 30 minutes, or that the normal sensitivity to glucagon is rapidly

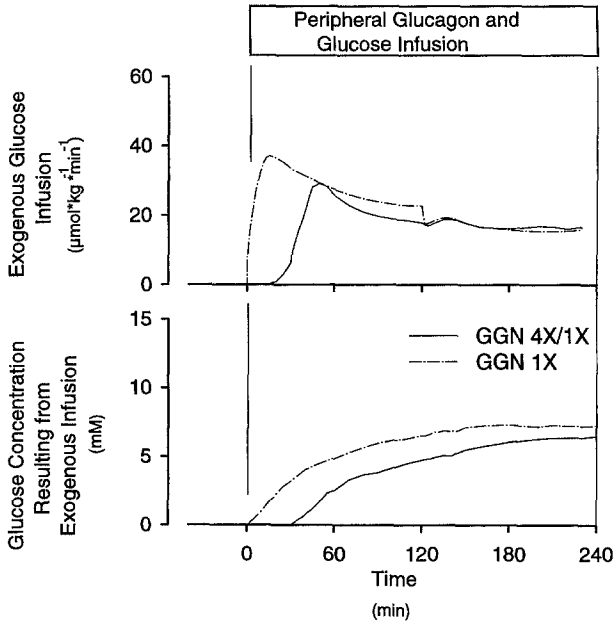

Fig 4. Exogenous peripheral glucose infusion rates for GGN 4X/1X and GGN 1X protocols and glucose concentration originating from exogenous infusion as determined by integration. Glucose was infused to match the levels noted in GGN 4X. Values are the mean ± SE.

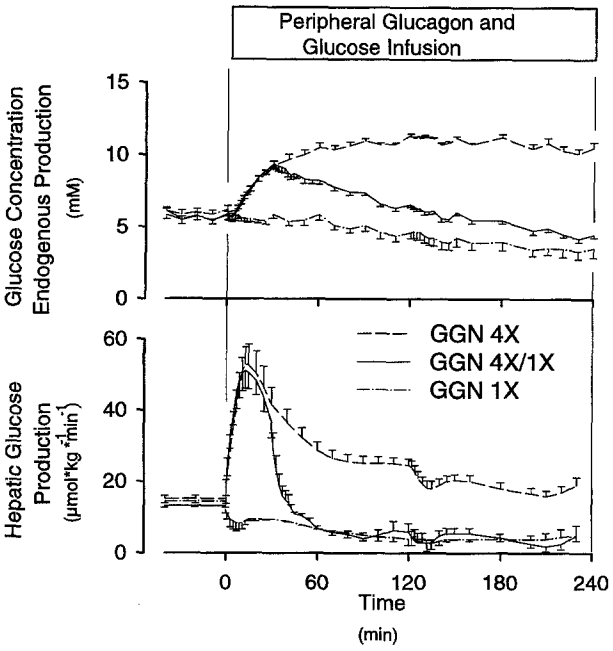

Fig 5. Glucose concentration from endogenous production and HGP during 4 hours of hyperglycemia induced by peripheral glucagon and glucose infusions. The GGN 4X group (n = 5) received peripheral glucagon at 4 ng · kg^{-1} · min^{-1} for 4 hours. The GGN 4X/1X group (n = 5) received 4 ng · kg^{-1} · min^{-1} glucagon for 30 minutes and then basal glucagon (1 ng · kg^{-1} · min^{-1}) for 3.5 hours. The GGN 1X group (n = 3) received basal glucagon for 4 hours. The latter 2 groups were infused with glucose to match the levels noted in GGN 4X. Values are the mean ± SE.

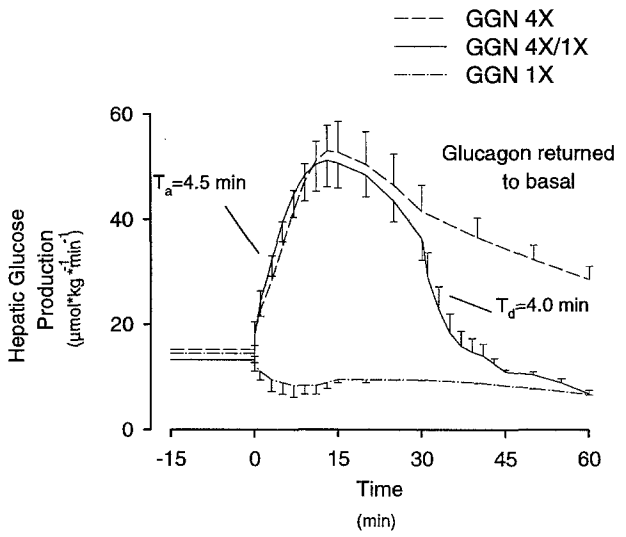

Fig 6. HGP during the first hour of hyperglycemia induced by peripheral glucagon and glucose infusions. The GGN 4X group (n = 5) received peripheral glucagon at 4 ng · kg^{-1} · min^{-1} for 4 hours. The GGN 4X/1X group (n = 5) received 4 ng · kg^{-1} · min^{-1} glucagon for 30 minutes and then basal glucagon (1 ng · kg^{-1} · min^{-1}) for 3.5 hours. The GGN 1X group (n = 3) received basal glucagon for 4 hours. The latter 2 groups were infused with glucose to match the levels noted in GGN 4X. Values are the mean ± SE.

recovered as hormone concentrations return to baseline. The latter explanation is consistent with an in vitro study indicating that hepatic glucagon receptors rapidly regain their normal affinity for glucagon when hormone concentrations are reduced following 30 minutes of hyperglucagonemia.[42] It is not consistent with the hypothesis that depletion of glycogen causes desensitization to glucagon action, because the glycogen consumed during hyperglucagonemia could not be rapidly repleted.

These data indicate a trend toward a smaller increment in glucose utilization (≈5 μmol · kg^{-1} · min^{-1}) when hyperglycemia is induced by increased glucagon as opposed to glucose infusion and basal glucagon replacement. Complementary studies from our laboratory support this finding. Hyperglycemia occurring in the absence of changes in insulin or glucagon has been shown to increase the absolute rate (not net) of glucose uptake by the liver.[47] Glucagon has been shown to block net hepatic glucose uptake under hyperglycemic, euinsulinemic conditions.[48] It therefore seems likely that glucagon blocked the ability of hyperglycemia to promote glucose flux into the liver in the present study.

In summary, this is the first set of experiments specifically designed to measure in vivo glucagon stimulation of HGP over intervals of 1 to 2 minutes. These data demonstrate that a step increase in glucagon results in rapid stimulation of HGP, reaching half-maximal stimulation in just 4.5 minutes. A step decrease in glucagon after 1 half-hour results in an equally rapid decline in HGP to the baseline seen in hyperglycemic controls. In comparison, studies in humans[49] and dogs[50] indicate that insulin action has a slow component and a more rapid component. Following an increase or a decrease in insulin, the maximal effect on glucose utilization and HGP is reached at about 1 hour. However, there is a more rapid initial change in glucose production that can be seen in 15 to 30 minutes.[50,51] Since both the α cell and β cell are exquisitely sensitive to changes in plasma glucose concentration, with changes in hormone secretion detectable with a 6-mg/dL and 3-mg/dL increase or decrease in glucose, respectively,[52] it appears that both glucagon and insulin are important for minute-to-minute glucose homeostasis. The rapid and sensitive hepatic response to glucagon makes this hormone well-suited for providing a burst of glucose in response to even a slight decrease in the plasma glucose concentration.

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