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Rates of Removal of Exogenous and Endogenous Glucagon from the Plasma of Sheep *in vivo*

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Abstract

The removal of exogenous and endogenous glucagon from plasma was determined *in vivo* in sheep weighing 53±1 (mean±s.e.) kg. Porcine glucagon was infused intravenously for 90 min. The metabolic clearance rates (MCR) were determined from plateau immunoreactive glucagon (IRG) concentrations in plasma and infusion rates of glucagon. The mean clearance rate (±s.e.) was 16·7±1·6 litres per hour ($n = 20$). Upon termination of the infusion, the decrease in IRG concentrations in plasma was determined. Least-squares regression analysis of non-linear functions indicated the data fit a two-component exponential function. The time constant for the rapid component of the plasma IRG disappearance function was $-0{\cdot}32 \pm 0{\cdot}04$ min^{-1} (mean±s.e.). The time constant for the slow component was $-0{\cdot}022 \pm 0{\cdot}008$ min^{-1}. The rate of removal of endogenous glucagon was estimated during the infusion of somatostatin when glucagon secretion was inhibited. The time constants (mean±s.e., $n = 8$) for the decrease in IRG during somatostatin infusion were $-0{\cdot}42 \pm 0{\cdot}08$ and $-0{\cdot}003 \pm 0{\cdot}002$ min^{-1} for fast and slow components, respectively. The time constants for the rapid components of exogenous and endogenous glucagon were not significantly different. This suggests that endogenous and exogenous glucagon are similarly removed from plasma.

Introduction

Little is known about the removal of glucagon from the plasma of ruminant animals. One study (Brockman *et al.* 1976) showed that 7–10% of the glucagon is removed as it passes through the liver. This is equivalent to removal of about one-third of the glucagon added to the portal vein. In the present study the fractional removal of exogenous and endogenous glucagon was studied. Porcine glucagon was infused for 90 min. After termination of the glucagon infusion, the rate of decline of immunoreactive glucagon (IRG) concentrations in plasma was determined. The removal of endogenous glucagon was determined from the decline of IRG in plasma during inhibition of glucagon secretion by infusion of somatostatin (SRIF).

Materials and Methods

Twelve healthy adult mixed-breed (predominantly Suffolk–Columbia) ewes, 53±1 kg (mean±s.e.) in weight, were used in this study. Eight sheep were used in the glucagon infusion experiments and in the SRIF infusions, although not all animals were used in each group. They were fed a maintenance diet of alfalfa pellets (400 g twice daily). Water and salt blocks (99% NaCl, 0·007% iodine and 0·004% cobalt by weight) were provided *ad libitum*. When experiments were conducted, feed was withheld for 24 h. This avoided infusion of glucagon during the post-feeding period when plasma glucagon concentrations normally are changing (Bassett 1972). All sheep had polyvinyl catheters implanted surgically into a mesenteric vein tributary and femoral vein and femoral artery at least 1 week before experimentation.

Glucagon (lot No. 258-V016-36, Lilly Research Laboratories, Indianapolis, Indiana), dissolved in sterile, pyrogen-free 0·85% (w/v) NaCl containing 0·2% (w/v) ovine serum albumin (fraction V powder, Sigma Chemical Co., St Louis, Missouri), was infused via the mesenteric vein catheter for 90 min at rates of 10, 15, 25 and 40 μg/h. In addition, three sheep were infused at 2·6 and 5·2 μg/h. In separate experiments with these three sheep SRIF was infused for 60 min before and during the glucagon infusion at these low rates. SRIF (lot No. AY-24910, Ayerst Laboratories, St Laurent, Quebec) was prepared in sterile saline with 100 mg of lactose as carrier per 500 μg of SRIF and infused for 90 min at 100 μg/h after a priming dose of 50 μg through the femoral vein catheter.

In the first set of experiments glucagon was infused into eight sheep. Blood samples were taken from the arterial catheters at 15-min intervals with the first sample taken 45 min before initiation of the glucagon infusion. Plateau IRG concentrations were reached after 30 min of infusion. The mean of the last three samples was taken to represent the plateau concentration. Blood samples were taken at 1, 2, 3, 6, 9, 12, 15, 21 and 30 min after termination of the glucagon infusion. For the experiments in which glucagon was infused at 2·6 and 5·2 μg/h, samples were taken only during pre-infusion and infusion periods. Thus, in these experiments, the rate of decrease in plasma IRG concentrations was not determined. In the second set of experiments, SRIF was infused into eight sheep, but not all the sheep in this group were the same as those used above. Three samples were taken before SRIF was infused. Additional samples were taken at 1, 2, 3, 5, 8, 12, 18, 24 and 30 min after SRIF infusion was initiated.

All blood samples were taken in heparinized syringes and immediately placed into tubes chilled on ice. The blood was centrifuged at 0–4°C and the plasma transferred into tubes containing 500 kallikrein inactivating units of aprotinin [Trasylol[R], Boehringer-Ingleheim (Canada) Ltd, Dorval, Quebec] and 3 mg of dipotassium EDTA per millilitre of plasma. These were stored at −20°C until analysed.

Glucagon concentrations were determined by radioimmunoassay as described in detail in Brockman (1979*a*). The sensitivity of the assay was 20 ng/l and intra- and interassay repeatabilities were 7±2% (n = 10) and 16±3% (n = 11) respectively. Glucose was estimated with glucose oxidase (Glucostat, Worthington Biochemical Corpn, Freehold, New Jersey).

Glucagon concentrations for calculation of the rates of removal or disappearance from plasma were normalized by expressing the values as a fraction of the plateau concentrations obtained during glucagon infusions. In those experiments in which SRIF was infused, the values were expressed as a fraction of the mean of the preinfusion values. This normalization facilitated graphical presentation of values as means ± standard errors. These normalized data, after parameter estimation by 'curve-stripping' (Franklin and Newman 1973), were subjected to non-weighted and weighted least-squares analyses for non-linear exponential functions (Brown 1977) to fit the form

$$q(t) = \sum_{i=1}^{n} q_i(\exp \lambda_i t),$$

where $q(t)$ is the plasma concentration as a fraction at time t, q_i and λ_i are constants, n, the number of exponents, and i, the exponent number. The weights were 1 and the reciprocal of the square of the observations for non-weighted and weighted analyses, respectively. Data from pooled experiments as well as individual experiments were used in regression analyses.

The half times were determined for each experiment from the following relationship:

$$(T_{0\cdot5} = (\ln 2)/\lambda.$$

The metabolic clearance rates (MCR) of glucagon, R, in litres per hour, were calculated from

$$R = I/(P-p),$$

where I is the infusion rate of glucagon in micrograms per minute and P and p the plateau and basal concentrations of glucagon respectively, in micrograms per litre.

The values of the exponents of endogenous and exogenous glucagon were evaluated by fitting of the functions to the data after simultaneous least-squares analysis on both sets of data with the constraint that the exponents of the fast components be equal. Significance was evaluated by F ratio test (Boxenbaum *et al.* 1974).

Results

The basal IRG concentrations in plasma ranged from 100 to 330 pg/ml. During glucagon infusions, the concentratons were increased up to 3300 pg/ml. The relationship between the changes in IRG concentrations and infusion rates of glucagon is presented in Fig. 1. The IRG increment was directly proportional to the infusion rate.

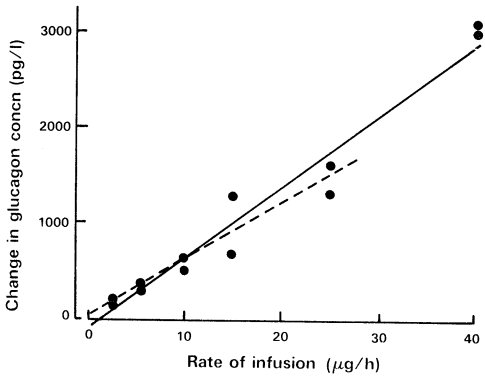

Fig. 1. Changes in glucagon concentrations in plasma of sheep during 20 intravenous infusions of glucagon at six different rates (—). Points at 2·6 and 5·2 μg/h represent six values at each rate. The regression equation is $y = 74x - 90$ ($r = 0{\cdot}98$) and the intercept and slope were -90 ± 51 and 74 ± 3 (means±s.e.), respectively. The broken line represents the regression when the values at 40 μg/h are excluded (slope = 61 ± 4, intercept = -3 ± 44). The regressions are not significantly different from each other.

The normalized results obtained during glucagon and SRIF infusion experiments are presented in Fig. 2. The removal of exogenous and endogenous glucagon fit two-term exponential functions. The addition of a third term in both cases did not reduce the sum of the squared deviations (Boxenbaum *et al.* 1974). The parameters

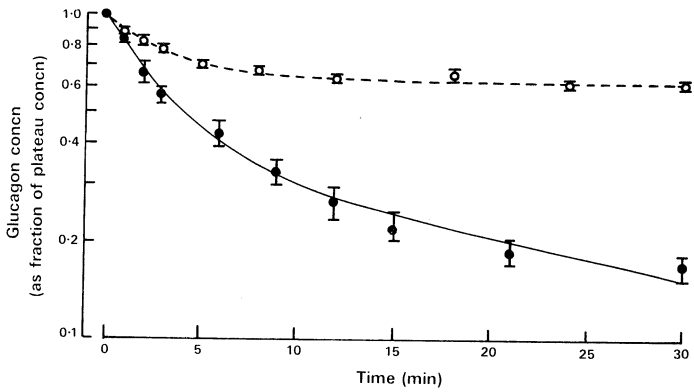

Fig. 2. Semilogarithmic plots of the decrease in IRG from plasma of sheep after termination of infusion of exogenous glucagon (●) or during the infusion of SRIF (○). Values are means±s.e. of eight experiments and represent concentration data normalized as a fraction of the zero time concentrations. The regression lines are those predicted from least-squares fit for non-linear exponential functions on pooled data.

of the functions (means of regression analysis of individual experiments) are presented in Table 1. The larger exponents of the exogenous and endogenous glucagon were not different as judged by a joint fit of both sets of data with the constraint that these exponents be equal (F ratio test, Boxenbaum *et al.* 1974).

The data were analysed using both non-weighted and weighted least-squares regression analyses. The use of weighting did not alter the sums of the squared deviations nor the values of the parameters significantly.

Table 1. Parameters of the two-component exponential equations for removal of glucagon from plasma of sheep after termination of glucagon infusion (exogenous glucagon) or infusion of SRIF (endogenous glucagon)

Means±s.e. are given for regression analyses from eight indivdual determinations

Parameter	Glucagon infusion	SRIF infusion
q_1	0·66 ±0·07	0·32 ±0·04
λ_1 (min^{-1})	−0·32 ±0·04	−0·42 ±0·08
q_2	0·34 ±0·07	0·67 ±0·04
λ_2 (min^{-1})	−0·022±0·008	−0·003±0·002
$t_{0\cdot5}$ (min)		
Fast	2·43 ±0·34	1·91 ±0·23
Slow	23 ±17	704 ±384

The time constants of the rapid terms were not significantly different between groups (F ratio test, Boxenbaum *et al.* 1974). The means±s.e. derived from individual experiments were −0·32±0·04 and −0·42±0·08 min^{-1}, respectively. The means of half times calculated individually were 2·43±0·34 and 1·91±0·23 min for exogenous and endogenous glucagon, respectively.

Table 2. Concentrations of IRG in plasma before hormone infusions and during IRG infusion and the incremental increase during infusion of glucagon

Infusion rate (μg/h)		IRG concentration (ng/l)			Glucose concentration (mM)	
Glucagon	SRIF	Basal	SRIF	Increment	Basal	Increment
2·6	—	232±20[A]	—	153±32[B]	3·3±0·2	0·11±0·06
2·6	100	212±32	90±20	150±23	3·2±0·1	0·16±0·07
5·2	—	173±35	—	320±37	3·5±0·2	0·44±0·06
5·2	100	180±35	95±15	290±30	3·4±0·2	0·56±0·06

[A] Values are means±s.e. of three experiments.
[B] Increments are the difference between basal and glucagon infusion concentrations and between SRIF infusion values and SRIF plus glucagon. In these experiments where both hormones were infused, the SRIF infusion was started 60 min before glucagon.

The MCR was 16·7±1·6 litres per hour (mean±s.e., n = 20). Table 2 presents data which suggest that infusion of glucagon at low doses does not suppress the secretion of endogenous glucagon.

Discussion

In this study, infusion of unlabelled glucagon was used to evaluate glucagon kinetics in sheep. Labelled hormone was not used since other studies with insulin indicate that the metabolism of labelled hormones may differ from that of unlabelled hormones (Genuth 1972; Sherwin *et al.* 1974). The infusion of exogenous hormone, particularly at the higher rates (40 μg/h), resulted in concentrations of IRG in plasma

of 3300 pg/ml. These values were higher than are ordinarily seen in sheep plasma. However, for the infusion rates used in this study the increase in IRG concentration in plasma was directly proportional to the infusion rate of glucagon (Fig. 1). The intercept (-90 ± 51, mean$\pm$s.e.) was not significantly different from zero. When the values from 40 μg/h infusion were excluded, the regression line obtained was not significantly altered. This is consistent with the fractional removal of glucagon from plasma being constant over a wide range of concentrations including those in excess of physiological values. A similar relationship has also been shown to occur in man (Fisher *et al.* 1976).

The possibility of cross-reaction with degradation products of glucagon in the immunoassay also exists. This would result in an overestimation of the glucagon concentrations and underestimation of its rate of removal.

Infusion of SRIF at 100 μg/h inhibits the secretion of pancreatic glucagon in sheep (Brockman 1979*b*). Since the plasma IRG was reduced to approximately 60% of basal values the secretion of IRG probably was not completely inhibited. This indicates that the dosage may have been relatively low. Others (De Vane *et al.* 1974; Dobbs *et al.* 1975) have obtained greater depressions of glucagon concentrations with larger doses in other species. Furthermore, some of the remaining plasma IRG may represent intestinal glucagon. Its basal concentrations in plasma do not appear to be depressed by SRIF (Marco *et al.* 1977) although SRIF may inhibit glucose-stimulated secretion of gut glucagon.

The time constants for the fast components were not significantly different between exogenous ($-0 \cdot 32 \pm 0 \cdot 04$ min^{-1}) and endogenous ($-0 \cdot 42 \pm 0 \cdot 08$ min^{-1}) glucagon. This is not surprising since it is unlikely that the porcine glucagon differs in composition from ovine glucagon. Sequencing studies indicate that mammalian glucagons may be identical (Bromer *et al.* 1971; Sundby and Markussen 1971).

The present study shows that the fractional removal of glucagon from plasma of sheep is similar to that reported in other species. The time constants for the fast components of glucagon disappearance determined for man (Assan 1972; Alford *et al.* 1976), dogs (Tanaka *et al.* 1979) and swine (van Hoorn *et al.* 1978) ranged from $0 \cdot 14$ to $0 \cdot 39$ min^{-1}.

MCR were calculated on the assumption that the basal secretion of glucagon was not depressed by glucagon infusion. This assumption may be incorrect. The hyperglycaemia associated with exogenous glucagon infusion may depress endogenous glucagon secretion (Brockman 1977). However, if endogenous glucagon secretion was reduced as much as 50%, the failure to include this in the calculation of MCR during infusion of glucagon at high dosages (40 μg/h) would overestimate the MCR by about 5% (basal concentration of 200 ng/l *v.* incremental increase of 3000 ng/l). At lower infusion rates this assumption would result in a larger overestimation of MCR if suppression of endogenous glucagon secretion occurred. However, at these infusion rates the hyperglycaemic stimulus to inhibit glucagon secretion would have been minimal since glucose concentrations increased only slightly (Brockman 1977). This contention is supported by data presented in Table 2. The increment in plasma glucagon associated with glucagon infusions in normal sheep and sheep whose basal glucagon concentrations were depressed by SRIF were the same at low rates of infusion of glucagon. Nevertheless, a small depression of basal secretion would not be apparent in the data in Table 2 since the analytical error for IRG is 16% (Brockman 1979*a*).

The MCR calculated for sheep (16·7±1·6 l/h, mean±s.e.) is similar to that reported for man (Alford *et al.* 1976). However, values reported for swine were approximately eightfold greater (van Hoorn *et al.* 1978).

The basal delivery of glucagon can be calculated as the product of MCR and basal IRG concentrations in plasma from data presented here. Assuming that within the concentration range of IRG in plasma of this study the removal of glucagon is directly proportional to the plasma concentration, and using concentrations and MCR calculated from incremental values presented in Table 2, the basal secretory rate of IRG would be 3·4±0·6 μg/h (mean±s.e.). This is not significantly different from 5·5±0·8 μg/h (mean±s.e.) which was determined from venoarterial concentration differences across the splanchnic viscera and splanchnic plasma flow (Brockman *et al.* 1976).

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