C-Peptide and Insulin Secretion

Relationship between Peripheral Concentrations of C-Peptide and Insulin and their Secretion Rates in the Dog

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Abstract. Estimation of the insulin secretory rate from peripheral C-peptide concentrations depends upon the following characteristics of C-peptide kinetics: (*a*) equimolar secretion of insulin and C-peptide by pancreatic beta cells; (*b*) negligible hepatic extraction of C-peptide; (*c*) constant metabolic clearance rate (MCR) of C-peptide over a physiological and pathophysiological range of plasma levels; and (*d*) proportional changes in the secretion rate of C-peptide and its peripheral concentrations under varying physiological conditions.

In the present experiments, the relationship between a variable intraportal infusion of C-peptide and its concentration in the femoral artery was explored in 12 pancreatectomized dogs. As the infusion of C-peptide was rapidly increased, the magnitude of its peripheral concentration initially increased less than the infusion rate by 20–30%. After an equilibration period of ~30 min, however, further increases and decreases in the intraportal infusion were accompanied by nearly proportional changes in its peripheral concentration. Estimates of the amount of C-peptide infused during the experiment based on the steady state C-peptide MCR and its peripheral concentration were within 20% of the amount of C-peptide actually infused. These experiments demonstrate that the portal delivery rate of C-peptide can be calcuated from its MCR and peripheral concentration in the dog. They also provide a basis for testing the validity of more complicated models of insulin secretion based on peripheral C-peptide concentrations in the dog as well as other species, including man.

Finally, we have shown that the hepatic extraction of endogenously secreted C-peptide is negligible in the basal state (3.1±6.1%), and does not change after oral glucose ingestion. The MCR of exogenous dog C-peptide was similar whether measured by constant peripheral intravenous infusion (12.3±0.7 ml/kg per min), constant intraportal infusion (13.4±0.6 ml/kg per min), or analysis of the decay curve after a bolus injection (13.5±0.7 ml/kg per min).

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Introduction

Insulin secretion rates cannot be directly calculated from peripheral insulin concentrations because of the large and variable hepatic extraction of the hormone (1–6). Since C-peptide is cosecreted with insulin on an equimolar basis from the pancreatic beta cell (7), it has been suggested that peripheral C-peptide concentrations may more accurately reflect changes in insulin secretion than do peripheral insulin concentrations. In support of this hypothesis, we have recently demonstrated that the hepatic extraction of dog C-peptide is negligible under basal steady state conditions as well as during an intravenous glucose infusion (8). Furthermore, the metabolic clearance rate of dog C-peptide is constant over a wide range of physiological plasma concentrations. On the basis of this information, the peripheral concentration of C-peptide may be expected to change in proportion to its secretion rate. This assumption has been made in a series of studies in man in which the secretion rate of C-peptide, and therefore insulin, was calculated as the product of its plasma concentration and metabolic clearance rate (9, 10). More complicated mathematical approaches to calculating insulin secretion rates based on peripheral C-peptide behavior have also been proposed (11, 12).

The validity of such indirect mathematical techniques for quantitating insulin secretion has not been tested experimen-

tally. Furthermore, in various studies, the metabolic clearance rate (MCR)[1] of C-peptide has been calculated by the constant infusion technique (8) as well as by analysis of decay curves following single bolus injections (13). The effect of these different methodological approaches on the calculated MCR is not known. The present study was therefore undertaken to examine the relationship between changes in the intraportal delivery rate of C-peptide and its concentration in the peripheral circulation. Different techniques for measuring the C-peptide MCR were also compared.

Methods

All experiments were performed in conscious mongrel dogs of both sexes (weight, 15–25 kg) after an overnight fast. The experiments were designed to investigate four specific areas related to the secretion and kinetics of dog C-peptide:

Metabolic clearance rate of C-peptide and insulin. This parameter was measured by three different techniques in the same animal: constant intraportal infusion, constant peripheral infusion, and analysis of the C-peptide decay curve following bolus injections.

Peripheral concentrations of exogenous dog C-peptide and insulin during their controlled intraportal infusion under nonsteady state conditions. The animals studied with this experimental protocol were the same animals in whom metabolic clearance rates of C-peptide and insulin had previously been measured (see above). This allowed for the calculation of an intraportal infusion rate (product of the peripheral C-peptide concentration and MCR) and its comparison with the actual intraportal infusion rate.

Hepatic extraction of C-peptide following oral glucose.

Secretion rate of endogenous dog C-peptide under basal fasting conditions.

The methods used in each group of experiments will now be described in detail.

Metabolic clearance rate of C-peptide and insulin

The MCR of dog C-peptide and porcine insulin, which has the same structure as dog insulin, was measured in 12 animals who had undergone total pancreatectomy to eliminate endogenous beta cell secretion 3 wk previously (as described in greater detail below). Three different techniques were compared in each animal.

Analysis of C-peptide and insulin concentration curves following peripheral intravenous bolus injections. Intravenous bolus injections of dog C-peptide (50–70 μg: 28.7–31.7 nmol) and insulin (0.6 U: 4.1 nmol)[2] were administered to 12 pancreatectomized dogs. The dog C-peptide used in these and subsequent experiments was isolated and purified from frozen dog pancreases as previously described (8). Preliminary experiments showed that the MCR of C-peptide was independent of the quantity of peptide in the bolus. The dose of insulin was chosen so that the peak plasma level would not exceed the concentration at which insulin clearance shows evidence of saturation. After the bolus injection, samples for C-peptide and insulin were drawn from the femoral artery at 1-min intervals for 5 min and then at 7, 9, 11, 14, 17, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min. Decay curves constructed from the plasma concentrations in each experiment were resolved by computer into (*a*) a single exponential, and (*b*) the sum of two exponentials by the least squares method. The adequacy of the fitted curve by these two techniques was compared using the approach described by Gallant (14).

The MCR was calculated as the ratio of the amount of peptide in each bolus (picomoles) to the area under the fitted decay curve integrated to infinity as previously described (13). Half disappearance times of C-peptide were calculated for each exponential as the ratio of the natural logarithm of 0.5 to the slope of the corresponding exponential. Volumes of distribution of C-peptide and insulin were calculated as the ratio of the quantity of peptide in the bolus (picomoles) to the sum of the intercepts of each exponential on the vertical axis (15).

Steady state peripheral intravenous infusion. The animals who were given the bolus injections of C-peptide and insulin described above also received peripheral intravenous infusions of dog C-peptide (3.4–6.8 pmol/kg per min) and insulin (3.4–6.8 pmol/kg per min), which were administered simultaneously. Femoral artery C-peptide and insulin concentrations were measured 25, 30, and 35 min into each infusion. Preliminary experiments revealed that steady state concentrations had been achieved by this time. Mean steady state C-peptide and insulin concentrations were calculated from concentrations at the three sampling times, and the steady state MCR for each peptide was calculated by dividing the infusion rate by the mean femoral artery concentration (16).

Steady state intraportal infusion. In these experiments the animals were given the same infusions by the intraportal route as they had received by the peripheral route. C-peptide and insulin were infused intraportally at a constant rate for 60 min. Mean steady state peripheral concentrations of C-peptide and insulin were calculated from the levels in the femoral artery at 40, 50, and 60 min into each infusion. The MCR was again calculated as the ratio of the infusion rate to the mean femoral artery concentration. This allowed the MCR measured by peripheral and intraportal routes of infusion to be compared. Using this technique, differences in the MCR by the two routes of administration can be assumed to be due to hepatic extraction of the peptide on its first pass through the liver.

The 12 dogs in which C-peptide and insulin metabolic clearance rates were measured were also studied according to the protocol described below.

Measurement of peripheral concentrations of C-peptide and insulin during their controlled intraportal infusion

The accurate measurement of the secretion rate of endogenously secreted C-peptide is difficult due to streaming effects in the portal vein and the pulsatile nature of spontaneous beta cell secretion. To regulate and more accurately quantitate the intraportal C-peptide delivery rate, a model was developed in which endogenous C-peptide secretion was eliminated by total pancreatectomy, and C-peptide and insulin were infused intraportally in controlled fashion to simulate the changes in insulin and C-peptide secretion that occur after oral glucose ingestion. Laporotomy was performed in 12 mongrel dogs and all visible pancreatic tissue was removed. An infusion catheter was then inserted into the splenic vein and secured so that its tip lay just distal to the origin of the portal vein. The end of the catheter was placed in a subcutaneous pouch. During recovery from the surgical procedure the animals were maintained on daily exogenous insulin injections and four tablets containing pancreatic enzymes with each feed (Cotazyme Organon Pharmaceuticals, West Orange, NJ). The animals were studied 3 wk later after an overnight fast, 24 h after the last insulin injection.

1. *Abbreviation used in this paper:* MCR, metabolic clearance rate.
2. The molecular weights of dog C-peptide and insulin are 2,206 and 5,807, respectively.

Infusions of dog C-peptide and porcine insulin (3.4–6.8 pmol/kg per min) were prepared in 1% bovine serum albumin saline just before each experiment and administered with a variable rate four-channel infusion pump (Harvard Apparatus Co., Inc., S. Natick, MA). To increase the accuracy with which the C-peptide and insulin infusion rates could be calculated, saline was simultaneously run through the same pump and the volume was measured over specific time intervals at each pump setting. After a 60-min constant basal infusion, 25 g glucose was administered orally to each animal at 0 min. The infusion rate of each peptide was then progressively increased at 5-min intervals so that by 60 min the infusion rates of dog C-peptide and insulin were six- to eightfold faster than the base-line infusion rate. Between 70 and 190 min, the infusion rates were progressively decreased at 10-min intervals, and from 200 to 240 min, the base-line infusion rate was resumed. Blood samples for dog C-peptide, insulin, and glucose were drawn from the femoral artery at 5–10-min intervals throughout the experiment. To relate the intraportal infusion rate to the peripheral concentration of C-peptide and insulin, predicted concentrations for both peptides were calculated at successive sampling times during each experiment according to the formula: Predicted concentration at t min = concentration at 0 min × (infusion rate at t min/infusion rate at 0 min).

The calculation of these hypothetical predicted concentrations of C-peptide and insulin was therefore based on the assumption that the ratio of the predicted concentration at t min to the concentration at 0 min is exactly equal to the ratio of the infusion rate at t min to the infusion rate at 0 min.

Hepatic extraction of C-peptide following oral glucose

A knowledge of the extent to which C-peptide is extracted by the liver after oral glucose is important for the interpretation of the above experiments. Thus, using the model previously described for measuring hepatic C-peptide extraction (8), this question was addressed in 11 additional dogs. Experiments were performed after an overnight fast. After a base-line observation period, the animals were given 25 g glucose orally. Samples for C-peptide were drawn from the portal and hepatic veins and femoral artery during the 30-min base-line period and every 5–20 min for a total of 240 min after glucose ingestion. Hepatic plasma flow was measured using the indocyanine green technique, and hepatic C-peptide extraction was calculated as a percentage of hepatic C-peptide delivery (8, 16).

Secretion rate of endogenous C-peptide under basal fasting conditions

Five dogs were studied 3 wk after sampling catheters were placed in the femoral artery, and portal and hepatic veins as previously described (2). Hepatic plasma flow was measured by the indocyanine green technique and a 70:30 ratio of portal vein to hepatic artery flow was assumed (17). Blood samples for C-peptide were drawn every 10 min for a total of 120 min. At each sampling time, the secretion rate of C-peptide was calculated as the product of the portal/peripheral arterial concentration difference and portal vein plasma flow. At the end of each of these experiments the MCR of C-peptide was measured during a constant peripheral intravenous infusion. This allowed C-peptide secretion rates to be calculated from the product of the arterial C-peptide concentrations and MCR.

Sample collection

In all experiments involving sampling from the femoral artery, and portal and hepatic veins, the hepatic vein sample was drawn 25 s after the other two samples to allow for hepatic transit time (5). Blood samples for insulin were allowed to clot at room temperature and the serum was stored at −20°C until assayed. C-peptide and glucose samples were drawn into tubes containing 500 KIU/ml Trasylol and 1.2 mg/ml EDTA. Plasma was separated immediately at 4°C and stored at −20°C until assayed.

Analytical techniques and statistical analysis. Serum insulin was assayed by a double antibody technique (18). Plasma glucose was measured with a glucose analyser (Yellow Springs Instrument Co., Yellow Springs, OH). Dog C-peptide was measured using the radioimmunoassay previously described (8). The minimal detectable concentration of C-peptide as determined by the least concentration causing significant displacement ($P < 0.05$) of the tracer was 0.005 pmol/tube. Interassay variation was ±6%and intraassay variation was ±5%.

The significance of differences between group means was evaluated by the two-tailed paired or unpaired t test or by a repeated measures analysis of variance where appropriate. All results were expressed as mean ± SEM, and $P < 0.05$ was considered significant. Calculations were performed on an IBM 3081 computer (IBM Instruments, Inc., Danbury, CT) using the Statistical Analysis System Version 82.3 (SAS Institute Inc., Cary, NC).

Results

Experiment I: metabolic clearance rates of C-peptide and insulin (Fig. 1 and Table I)

The plasma concentrations of C-peptide after its bolus injection are shown in Fig. 1. The concentration curves were analyzed separately for each animal. In eight of the 12 animals, a statistically better fit of the data was obtained using a two- rather than a single-exponential model. In two animals the difference between the single and two-exponential analysis was

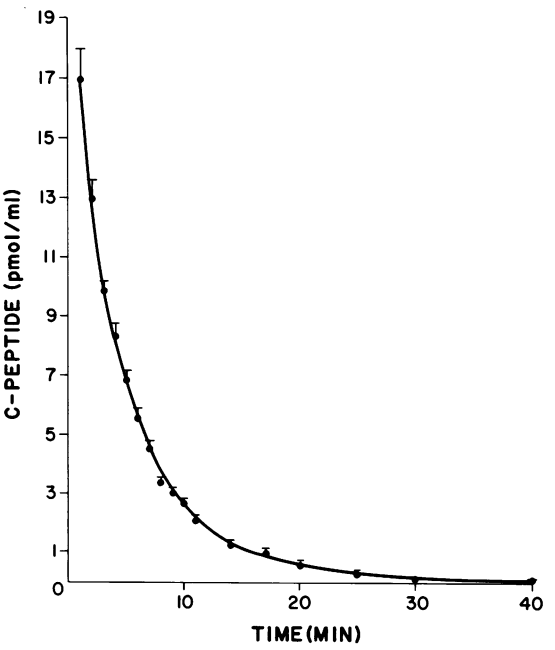

Figure 1. Concentrations of C-peptide in the femoral artery following peripheral intravenous bolus injections.

not significant, and in the remaining two dogs, it was possible to fit only a single exponential to the data. The MCR calculated when a single exponential was fitted to each decay curve (13.5±0.7 ml/kg per min) was greater than when a two-exponential model was used (12.9±0.7 ml/kg per min $P < 0.001$). This difference was due to the fact that the area under the curve fitted with two exponentials was greater than the area under a single exponential. The plasma half-lives of each exponential in the two-exponential models were 1.4±0.3 and 6.6±1.6 min, respectively (Table I). The MCR was derived from analysis of the decay curves, and did not differ from the MCR calculated by the constant infusion technique. The MCR calculated by the latter method was similar regardless of whether the constant infusion was administered via a peripheral vein (12.3±0.6 ml/kg per min) or intraportally (13.4±0.6 ml/kg per min). The volume of distribution of C-peptide was 1,035±74 ml.

A two-exponential model gave a better fit for the decay curve of insulin in all except one dog. The half-lives of the two exponentials were 1.1±0.2 and 6.3±1.3 min, respectively. In contrast to C-peptide, the MCR of insulin calculated by analysis of the decay curve (29.6±3.0 ml/kg per min) was significantly greater than the MCR during a constant peripheral infusion (18.5±0.8 ml/kg per min, $P < 0.02$). The MCR of insulin calculated by constant intraportal infusion (34.2±2.0 ml/kg per min, $P < 0.001$) was significantly greater than that by peripheral intravenous infusion, which is consistent with the important role played by the liver in the clearance of insulin.

Experiment II

Peripheral concentrations of C-peptide and insulin during their intraportal infusion (Fig. 2). C-peptide and insulin were infused intraportally in 12 dogs who had undergone total pancreatectomy (Fig. 2). A basal infusion of C-peptide (75.5±4.4 pmol/min) was administered for 60 min. Thereafter, at 0 min, the dogs were given 25 g glucose orally and the infusion rate progressively increased at 5-min intervals to reach a peak of 452.8±26.7 pmol/min between 60 and 70 min. The infusion rate was then reduced at 10-min intervals to return to the base-line infusion rate at 190 min. This infusion rate was continued until 240 min. The same pattern of change was followed with the insulin infusion. The plasma glucose concentration was 137±11 mg/dl during the basal infusion. It rose to a peak of 228±43 mg/dl at 50 min, and returned to base line 140 min after glucose. C-peptide and insulin concentrations measured in the femoral artery are also shown in Fig. 2. The C-peptide concentration at 0 min (0.28±0.02 pmol/ml) increased to reach a peak of 1.70±0.14 pmol/ml at 70 min and then fell progressively as the infusion rate was decreased. At 220 min the concentration was 0.30±0.01 pmol/ml. Insulin concentrations were three- to fourfold lower than those of C-peptide throughout the experiment, despite similar infusion rates.

Table I. Distribution Volume, Plasma Half-life, and MCR of C-Peptide and Insulin

	C-peptide	Insulin
Distribution volume (*ml*)	1,035±74	1,691±240
% body weight	5.3±0.3	8.9±1.5
Half-life (*min*): exponential 1	1.4±0.3	1.1±0.2
exponential 2	6.6±1.6	6.3±1.3
MCR (*ml/kg/min*):		
Bolus injection: single exponential	13.5±0.7	
two exponentials	12.9±0.7	29.5±3.0
Constant infusion: portal	13.4±0.6	34.2±2.0
peripheral	12.3±0.7	18.5±0.8

MCR of C-peptide was similar by the three methods used in its measurement, whereas the MCR of insulin was significantly lower ($P < 0.05$) during constant peripheral infusion than by the other two techniques.

The relationship between the intraportal infusion rate and the peripheral C-peptide and insulin concentrations was explored by calculating predicted concentrations of C-peptide and insulin throughout each experiment according to the formula defined in Methods. The mean predicted concentrations for C-peptide and insulin in relation to the concentrations measured by radioimmunoassay are shown in Fig. 3. Thus, the measured peripheral C-peptide concentrations were less than the predicted concentrations between 0 and 30 min, during the time that the infusion rate was rapidly increased. Between 30 and 190 min, however, the measured and predicted concentrations were very similar, the actual concentration being between 90 and 110% of the predicted concentration. As the infusion rate returned to base line, the actual concentration was temporarily greater than the predicted concentration (Fig. 3 *A* and *B*). In contrast, the measured peripheral insulin concentration was consistently less than that predicted until the infusion rate returned to base line (Fig. 3 *C* and *D*).

Prediction of the C-peptide infusion rate from its peripheral plasma concentration and MCR (Fig. 4; Table II). Since in separate studies the MCR of C-peptide had been measured in these pancreatectomized animals, the infusion rate of C-peptide could be calculated at individual time points in each experiment as the product of its peripheral plasma concentration and metabolic clearance rate. Furthermore, the effect of using MCR data derived by different techniques could be evaluated. C-peptide infusion rates calculated by these indirect techniques are shown in comparison to the actual infusion rates in Fig. 4. Between 0 and 30 min, the actual infusion rate could be predicted with 70–80% accuracy by these indirect techniques. Thereafter, the actual and calculated rates were similar, although use of the MCR derived from a two-exponential fit of the decay curves consistently underestimated the infusion rate. The total quantity of C-peptide actually infused between 0 and 240 min was compared with the corresponding value calculated from the peripheral concentration and MCR as shown in Table II. Estimates of the total quantity of C-peptide infused over this time based on the steady state MCR or the

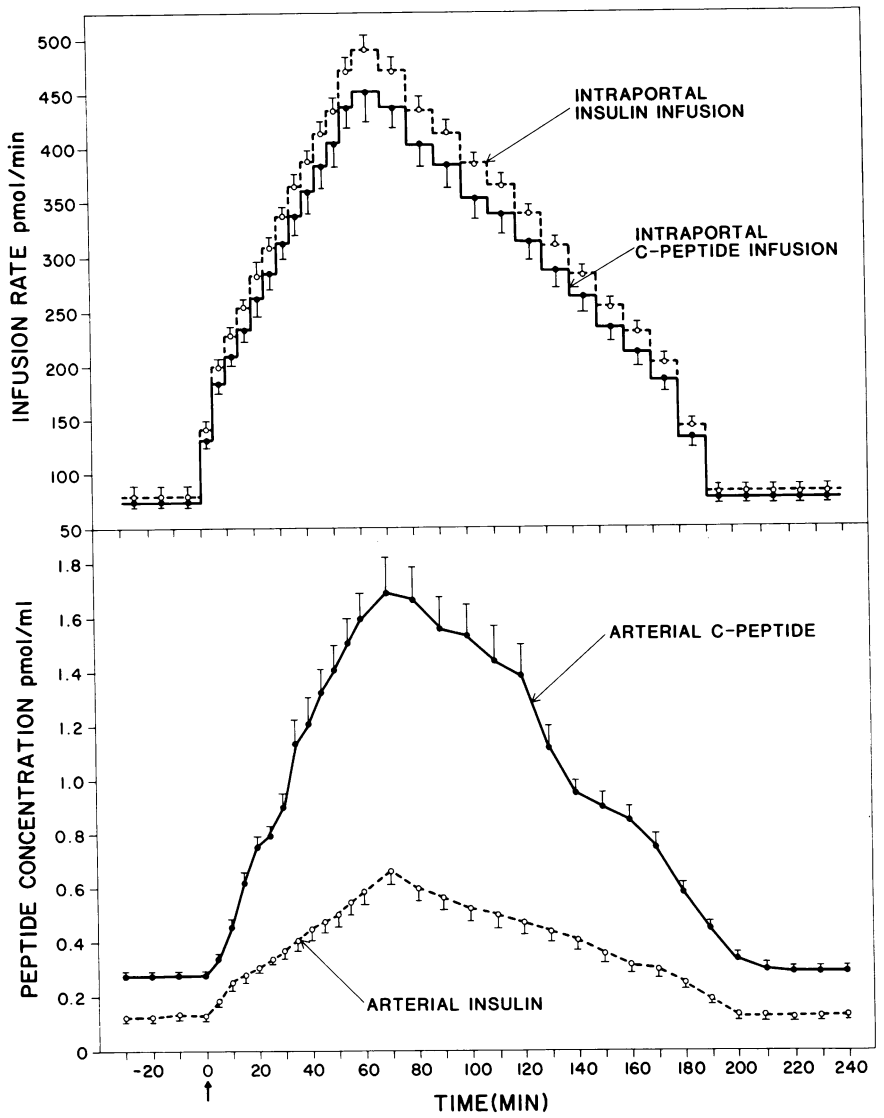

Figure 2. Peripheral concentrations of C-peptide and insulin during their intraportal infusion. Intraportal infusion rates of C-peptide (— ● —) and insulin (- - - ○ - - -) are shown in the upper panel. The simultaneously measured arterial concentrations of C-peptide (— ● —) and insulin (- - - ○ - - -) are shown in the lower panel. Before the ingestion of glucose at 0 min, C-peptide and insulin were infused intraportally for a total of 60 min. Data from the latter 30 min of this infusion are shown.

MCR derived from fitting a single exponential to the C-peptide decay curve did not differ from the amount of C-peptide actually infused. Estimates based on the MCR calculated from a two-exponential fit of the C-peptide decay curve were significantly lower than the amount of C-peptide actually infused by paired t test analysis ($P < 0.05$).

Experiment III: hepatic extraction of C-peptide after oral glucose ingestion

The above data suggested that the overall hepatic extraction of C-peptide was negligible during these experiments. However, since a transient increase in hepatic extraction could explain the findings immediately after glucose ingestion, the hepatic extraction of endogenously secreted C-peptide was measured before and after 25 g oral glucose.

The overall hepatic extraction in these dogs was very low (7.1±2.2%). During the base-line observation period, the value was 3.1±6.1%, and values of −2.2±5.7, 5.8±5.6, −1.2±5.5, 11.6±5.6, 7.2±6.7, 4.9±4.4, 5.0±14.1, 7.1±4.0, 5.4±4.8, 7.0±7.9, 16.0±2.7, 19.7±3.0, 11.3±5.6, 7.7±5.1, 7.6±4.9, −2.7±8.5, and 18.0±6.9 were measured at 5, 10, 15, 20, 25, 30, 45, 60, 80, 100, 120, 140, 160, 180, 200, 220, and 240 min after oral glucose. Repeated measures analysis of variance did not demonstrate any significant change in extraction as a result of the oral glucose.

Experiment IV: endogenous C-peptide secretion rates under basal steady state conditions

In these five animals, femoral artery and portal vein C-peptide concentrations were 0.20±0.04 and 0.289±0.05 pmol/ml, respectively, and did not change over the 120 min observation period. The calculated C-peptide secretion rates are shown in

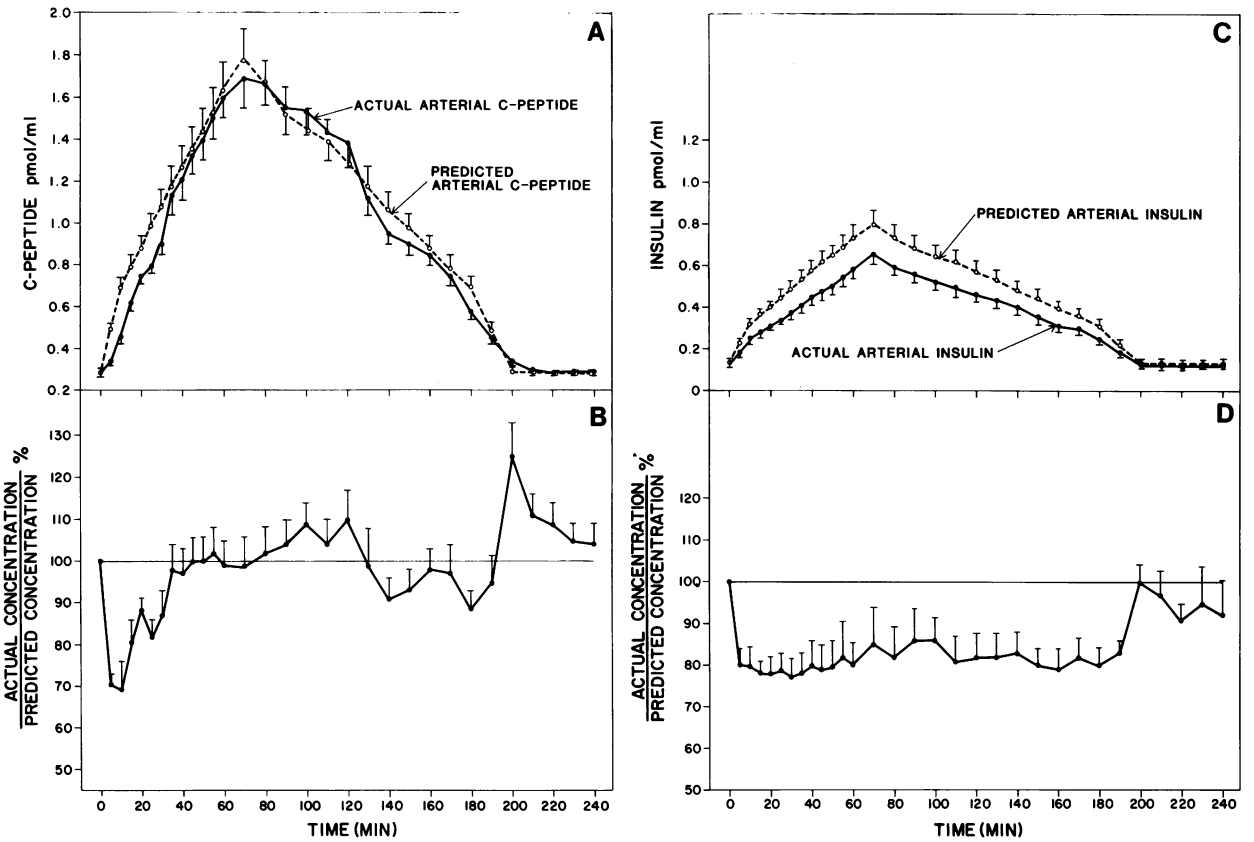

Figure 3. The relationship between the intraportal infusion rates of C-peptide and insulin and their peripheral concentrations. Measured (— ● —) and predicted (- - - o - - -) concentrations of C-peptide in the experiments described in Fig. 2 are shown in *A*. The predicted concentrations of C-peptide were calculated as described under Methods. The predicted concentration of C-peptide expressed as a percentage of the measured concentration is shown in *B*. The corresponding measured (— ● —) and predicted (- - - o - - -) concentrations of insulin are shown in *C*. The measured concentrations of insulin expressed as a percentage of the predicted concentrations are shown in *D*. All data points represent mean±SEM of data from the 12 experiments.

Table III. The C-peptide secretion rates in these animals were also calculated from the arterial concentration, and the MCR was determined during a constant peripheral infusion (Table III).

The C-peptide secretion rates calculated by the two methods were similar in three dogs. In two animals, however, the secretion rate, calculated from the MCR and arterial C-peptide concentration, was approximately twofold higher than the rate calculated by the direct portal sampling technique.

Discussion

Although the peripheral C-peptide concentration has been used as a marker of insulin secretion, the present studies represent the first direct in vivo demonstration that the secretion rate of C-peptide, and therefore insulin, can be calculated accurately from its peripheral concentration. The demonstration that the metabolic clearance rate of C-peptide is not altered by ingestion of oral glucose is an important experimental finding in validating the use of C-peptide as a marker of insulin secretion under different physiological conditions. In this regard a significant unanticipated result emerged from the present studies. As the intraportal infusion rate of C-peptide was rapidly increased, its peripheral concentration increased less than predicted, resulting in a consistent underestimation of that rate by approximately 30%. This observation may indicate that to measure a rapid increase in insulin secretion accurately at discrete time points (e.g., during a glucagon or intravenous glucose tolerance test), more complicated mathematical techniques for analyzing peripheral C-peptide concentrations may be needed as has been proposed by Eaton et al. (11).

C-peptide secretion rates have been calculated from peripheral plasma concentrations and the C-peptide MCR (9, 10). Since the accurate quantitation of the metabolic clearance rate of C-peptide is fundamental to this approach, different methods of calculating the C-peptide MCR were compared. The MCR calculated by analysis of the C-peptide decay curve

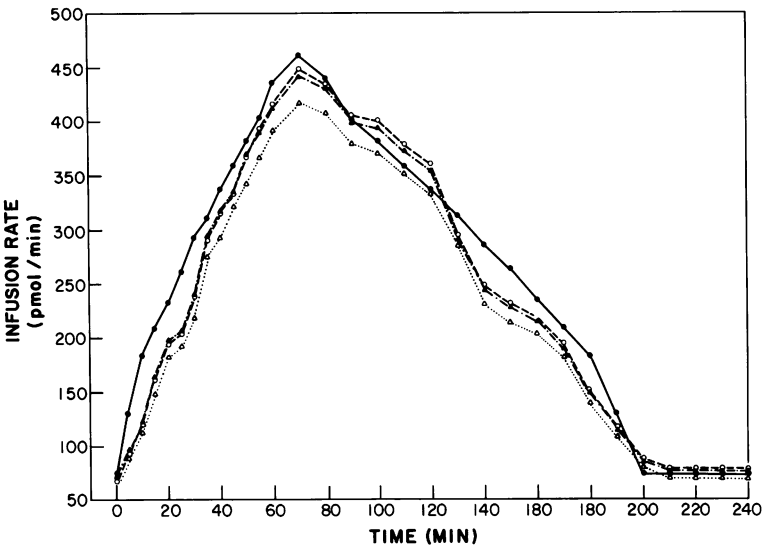

Figure 4. The intraportal infusion rate of C-peptide (— ● —) in comparison to estimates of this rate based on the product of the C-peptide MCR and femoral artery C-peptide concentration at each sampling time. Mean data for the 12 experiments are shown. The MCR used in these calculations was derived by constant intraportal infusion (- - - ○ - - -), fitting a single exponential to individual C-peptide decay curves (-·- ▲ -·-) and fitting two exponentials to individual C-peptide decay curves (· · · △ · · ·).

after bolus injection was similar to the MCR calculated during constant infusion of C-peptide given either intraportally (13.4±0.6ml/kg per min) or peripherally (12.3±0.7ml/kg per min). The similarity of the C-peptide MCR measured by peripheral and intraportal infusion provides convincing evidence for our previous findings that the liver does not play a significant role in the metabolic disposal of this peptide (8). In contrast, the MCR of insulin measured during its constant intraportal infusion (34.2±2.0 ml/kg per min) was greater than the MCR measured during a peripheral infusion (18.5±0.8 ml/kg per min). This is consistent with the major role of the liver in the metabolic disposal of insulin during its first pass

Table II. Calculation of Intraportal C-Peptide Infusions from Peripheral C-Peptide Concentrations

	A	B		C		D	
Dog no.	Actual infusion	Calculated infusion		Calculated infusion		Calculated infusion	
	(*nmol*)	(*nmol*)	%	(*nmol*)	%	(*nmol*)	%
6	60.457	57.857	95.7	61.116	101.1	57.857	95.7
7	42.856	43.853	102.3	57.253	133.6	51.467	120.1
8	42.859	38.148	89.0	37.026	86.4	31.696	74.0
9	71.676	91.738	128.0	74.096	103.4	68.047	94.9
10	62.441	50.181	80.4	46.098	73.8	42.544	68.1
11	67.060	54.350	81.0	71.836	107.1	69.946	104.3
12	57.887	46.722	80.7	48.965	84.6	43.358	74.9
13	75.773	77.819	102.7	68.840	90.9		
14	62.304	56.964	91.4	51.598	82.8	46.232	74.2
15	65.995	59.263	89.8	59.263	89.8	55.876	84.7
16	79.367	81.333	102.5	74.440	93.8		
17	61.652	56.746	92.0	59.583	96.6	56.341	91.4
Mean±SEM			94.6±3.8		95.3±4.4		88.2±5.1

Values in column A represent the amount of C-peptide infused between 0 and 240 min in the experiments shown in Fig. 2. Values in columns B, C, and D represent estimates of the amount of C-peptide infused over the same time interval in the same experiments. C-peptide infusion rates were calculated at each sampling time as the product of the C-peptide concentration and MCR, and the area under this curve was then calculated between 0 and 240 min. The MCR for the calculations in column B was derived by constant intraportal infusion; in column C, by fitting a single exponential to the decay curves following bolus injections; and in column D, by fitting two exponentials to the C-peptide decay curves. The missing values in column D were from two dogs in whom two exponentials could not be fitted to the decay curves. The values in columns B, C, and D are expressed as a percentage of the value in column A.

Table III. C-Peptide Secretion Rates in Five Dogs After an Overnight Fast

Dog no.	A	B
	pmol/min	*pmol/min*
1	39.0±6.8	41.4±4.1
2	26.5±1.8	45.4±3.4
3	18.6±2.4	34.7±1.4
4	64.5±12.7	70.8±1.6
5	31.7±2.5	32.7±2.5
Mean±SEM	36.1±7.8	45.0±6.8

Column A represents the secretion rate calculated as the product of the portal arterial C-peptide concentration difference and the portal plasma flow. Column B represents the secretion rate calculated as the product of the C-peptide MCR and femoral artery C-peptide concentration. Each value represents the mean±SEM of 12 measurements obtained at 10-min intervals over a period of 120 min in each animal.

through this organ. Of interest was the finding that the MCR of insulin calculated by analysis of its decay curve after peripheral intravenous bolus injections (29.5 ± 3.0 ml/kg per min) was greater than that calculated by constant peripheral intravenous infusion (18.5 ± 0.8 ml/kg per min). The reason for this difference is unclear, although it may be related to the fact that after a bolus injection, insulin appears to distribute in a volume (1,691 ± 240 ml) that is larger than the plasma volume and represents 8.9 ± 1.5% of the body weight. This large initial volume of distribution contributes to the very rapid initial decay of serum insulin concentrations seen after bolus injection, and may result in an overestimation of the MCR when measured by this technique compared with the constant infusion technique. This observation, which to our knowledge has not been published previously, should be considered when comparing the results of studies that have used different techniques for measuring insulin clearance. In contrast, the initial volume of distribution of C-peptide was 1,035±74 ml or 5.3±0.3% of the body weight, representing a volume approximately equal to the plasma volume.

In the present studies, the relationship between changes in the intraportal delivery rate of C-peptide and its peripheral arterial concentration was directly examined. To increase the accuracy with which the delivery of C-peptide could be quantitated, experiments were performed in dogs who had undergone total pancreatectomy to eliminate endogenous C-peptide secretion. C-peptide and insulin were infused intraportally in a pattern designed to simulate the changes in endogenous insulin secretion that occur after oral glucose ingestion. As the C-peptide infusion rate was rapidly increased, its peripheral concentration increased less than the infusion rate by 20–30%. After 30 min, however, the subsequent changes in the intraportal infusion rate were followed by parallel changes in the peripheral C-peptide concentration. In contrast, the peripheral concentration of insulin increased proportionately less than the intraportal infusion rate during both the increase and decrease in the intraportal infusion rate (Fig. 3). The reason for the discrepancy between the measured peripheral C-peptide concentrations and the predicted concentrations during the rapid increase in the infusion rate is not known. This finding could be because of a transient increase in hepatic C-peptide extraction. However, direct measurement of the hepatic extraction of endogenously secreted C-peptide revealed that the hepatic extraction of C-peptide was negligible in the fasting state, and no consistent increase was detected after oral glucose. It seems possible that as the C-peptide infusion rate increases, it distributes more rapidly into extravascular spaces and tissues because of a transient disequilibrium between different body compartments, and this results in a relatively lower plasma concentration. We favor this explanation of our findings, although we do not as yet have objective data to prove the hypothesis.

The present experiments, in which C-peptide was infused intraportally, provide the first direct experimental evidence that, at least in the dog, peripheral C-peptide can be used to calculate the C-peptide and therefore insulin-secretion rate. Although as the C-peptide infusion rate was rapidly increased, its peripheral concentration increased proportionately less than the infusion rate; this effect seemed transient, and once equilibration had occurred, further changes in the infusion rate were accompanied by similar relative changes in the peripheral C-peptide concentrations. Furthermore, estimates of the amount of C-peptide infused over longer time intervals derived as the product of the C-peptide MCR and plasma C-peptide concentration were within 20% of the amount of C-peptide actually infused. The extent to which these findings apply to the calculation of the secretion rate of human C-peptide is not known. Since human C-peptide has a longer plasma half-life and slower MCR than dog C-peptide, peripheral C-peptide concentrations may not reflect its secretion rate as accurately as in the dog. Nevertheless, the current data provide a foundation on which to test the accuracy of mathematical models of insulin secretion based on C-peptide behavior in the periphery.

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