Metabolism of C-Peptide in the Dog

IN VIVO DEMONSTRATION OF THE ABSENCE OF HEPATIC EXTRACTION

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A B S T R A C T The in vivo hepatic metabolism of connecting peptide (C-peptide) in relation to that of insulin has not been adequately characterized. A radioimmunoassay for dog C-peptide was therefore developed and its metabolism studied in conscious mongrel dogs, with sampling catheters chronically implanted in their portal and hepatic veins and femoral artery. The hepatic extraction of endogenous C-peptide under basal conditions was negligible (4.3±4.5%) and was similar to the hepatic extraction of C-peptide measured during the constant exogenous infusion of C-peptide isolated from dog pancreas. Simultaneously measured hepatic extraction of endogenous and exogenously infused insulin were 43.8±7.6 and 47.5±4.4%, respectively. The metabolic clearance rate of infused C-peptide was 11.5±0.8 ml/kg per min and was constant over the concentration range usually encountered under physiological conditions. In additional experiments, the effect of parenteral glucose administration on the hepatic extraction of C-peptide and insulin was investigated. The hepatic extraction of C-peptide (6.2±4.0%) was again negligible in comparison with that of insulin (46.7±3.4%). Parenteral glucose administration did not affect the hepatic extraction of either peptide irrespective of whether it was infused peripherally, intraportally, or together with an intraportal infusion of gastrointestinal inhibitory polypeptide. The fasting C-peptide insulin molar ratio in both the portal vein (1.2±0.1) and femoral artery (2.1±0.3) was also unaffected by the glucose stimulus. These results therefore indicate that, since the hepatic extraction of C-peptide is negligible and its clearance kinetics linear, the peripheral C-peptide concentration should accurately reflect the rate of insulin secretion. New approaches to the quantitation of hepatic extraction and secretion of insulin by noninvasive techniques are now feasible.

INTRODUCTION

Because of the large and variable hepatic extraction of insulin, peripheral insulin levels may not accurately reflect the concentration of insulin present in the portal vein (1–9). This has severely limited our ability to study insulin secretion in human subjects and controversy still persists concerning the importance of abnormalities of insulin secretion in the pathogenesis of Type II diabetes (10). Since connecting peptide (C-peptide)[1] and insulin are secreted in equimolar concentrations from the beta cell (11), it has been suggested that the peripheral concentration of C-peptide may more accurately reflect the portal insulin concentration than does the peripheral insulin concentration. If this is indeed true, it should be possible to develop models of the hepatic extraction and secretion of insulin based on peripheral C-peptide and insulin concentrations, thus enabling the study of these processes by noninvasive techniques. The validity of this use of the C-peptide concentration is dependent on the demonstration that its hepatic extraction is consistently

[1] *Abbreviations used in this paper:* C-peptide, connecting peptide; CPR, C-peptide immunoreactivity; GIP, gastrointestinal inhibitory polypeptide; ICG, indocyanine green; MCR, metabolic clearance rate.

negligible and its metabolic clearance rate linear under normal physiological conditions. Because of marked species differences in the C-peptide molecule (12), the accurate measurement of C-peptide in different animal species requires the development of specific radioimmunoassays. Because of the difficulties inherent in establishing such assays, a detailed study of the hepatic metabolism and metabolic clearance rate of C-peptide in relation to that of insulin has not been undertaken in an animal model, and the validity of the use of C-peptide as a marker of insulin secretion has not been adequately tested in vivo. To enable us to examine these questions directly, we developed a radioimmunoassay for C-peptide in the dog and studied its metabolism both under steady state conditions as well as following stimulation by intravenous glucose.

METHODS

Isolation of dog C-peptide and preparation of radioimmunoassay reagents. Frozen pancreas (2 kg, removed from anesthetized, normal mongrel dogs) was pulverized and homogenized in 85% (vol/vol) ethanol containing 0.1 M HCl (3 ml/g of tissue) using a Waring blender (Waring Products Div., Dynamics Corp. of America, Rochester, NY). The homogenate was stirred at 4°C for 4 h and was centrifuged at 8,000 g for 30 min to remove insoluble material. The supernatant fluid was collected and adjusted to pH 5 with ammonium hydroxide; 4 vol of ice-cold acetone was added, the solution was mixed, and peptides and proteins were allowed to precipitate during 48 h at 4°C. The supernatant was removed by aspiration and the precipitate (containing insulin and C-peptide) was collected, dried under a stream of nitrogen, and dissolved in 400 ml of 3 M acetic acid. The peptide solution was then gel filtered on a column (10 × 95 cm) of Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, CA) equilibrated with 3 M acetic acid; 24-ml fractions were collected. C-peptide, which emerged from the column in the latter half of the insulin peak and slightly beyond, was identified by paper electrophoresis by ninhydrin spray (13). Fractions containing the peptide were pooled, concentrated on a rotary evaporator, and applied to a column of SP-Sephadex C-25 (0.9 × 5 cm, sodium form, overlayered with 1 cm of Sephadex G-10 [Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ]) equilibrated with 3 M acetic acid. The C-peptide eluted slightly beyond the void volume of the column during isocratic elution with 3 M acetic acid; insulin and other contaminants were eluted with a linear gradient of NaCl in 3 M acetic acid (0–0.5 M). C-peptide-containing fractions identified by paper electrophoresis were pooled and dried. The peptide was finally dissolved in a small amount of water and precipitated with ethanol and ether for collection and storage at −20°C. The isolated material (15 mg) had the amino acid composition expected for dog C-peptide and is estimated to be >90% pure. It is important to note that contamination by insulin or proinsulin was not evident as assessed by amino acid analysis and radioimmunoassay. The peptide does not react in radioimmunoassays designed to detect human C-peptide.

Dog C-peptide isolated as described above was coupled to bovine serum albumin using difluorodinitrobenzene (Sigma Chemical Co., St. Louis, MO) as previously described (14) and the conjugate was used as the immunogen for the in-

duction of anti C-peptide antibodies in New Zealand white rabbits. Intradermal immunization using complete Freund's adjuvant was carried out by standard methods. Preparation of tyrosylated C-peptide for eventual iodination and use as the radioimmunometric tracer involved NH2-terminal derivatization with *tert*-butyloxycarbonyl-L-tyrosine N-hydroxysuccinimide ester (Bachem, Torrance, CA) (15). The C-peptide (1 μmol) was dissolved in 0.05 ml of 0.1 M N-ethylmorpholine acetate buffer, pH 8.5, and was treated with a 10-fold molar excess of the active ester dissolved in dimethyl formamide during 1 h at 4°C. The peptide was purified on a column of SP-Sephadex C-25 in 3 M acetic acid as described above, and the peptide fractions showing optical absorbance at 275 nm were pooled, dried, and lyophilized. The N-protected, tyrosylated peptide was iodinated by the chloramine T method and was purified by gel filtration on a column of Bio-Gel P-6 equilibrated with 1 M acetic acid. Pooled fractions containing *tert*-butyloxycarbonyl-L-[^{125}I]iodotyrosyl-C-peptide were dried under vacuum. The material was then dissolved in 1 ml of anhydrous trifluoroacetic acid (Pierce Chemical Co., Rockford, IL) and the solution was allowed to stand at 22°C for 2 h to effect removal of the *tert*-butyloxycarbonyl protecting group, a procedure that significantly increased tracer binding to the antibody. The trifluoroacetic acid was removed under a stream of nitrogen and the labeled peptide was dissolved in radioimmunoassay buffer. Dog C-peptide isolated from the same preparation was used for both the immunoassay standard and infusion studies described later.

Assay development and validation. The radioimmunoassay protocol is a modification of that used for the radioimmunoassay of human C-peptide in our laboratory (16). Plasma samples or dog C-peptide standards (0.1 ml) were incubated at 4°C for 48 h with our antiserum directed against dog C-peptide (R1 antibody—final dilution 1:10,000). Total assay volume was 700 μl and antiserum and tracer were diluted in 0.04 M phosphate buffer, pH 7.4, containing 0.1% bovine serum albumin. For preparation of C-peptide standards and for dilution of plasma, 0.04 M phosphate buffer containing isotonic NaCl and 6% albumin to mimic the protein concentration of plasma was used. Separation of bound from free C-peptide was achieved by the addition of 1.6 ml 95% ethanol to each assay tube followed by centrifugation at 3,000 rpm for 10 min. Total incubation time was 48 h and, since maximal assay sensitivity was achieved under nonequilibrium assay conditions, the tracer was added to the incubation mixture 24 h after the other reagents. The assay standard curve is shown in Fig. 1. The minimal detectable dose of C-peptide as determined by the least concentration causing significant displacement (*P* < 0.05) of the tracer was 0.005 pm/tube (1 pm of dog C-peptide = 2.206 ng). Interassay variation was ±6% and intraassay variation was ±5%. Recovery of C-peptide added to plasma over a concentration range of 0.08 to 0.5 pm/ml was 97.4±2.4% (*n* = 14). Insulin, glucagon, and pancreatic polypeptide did not cross-react with the antibody at concentrations of up to 1,000 pm/ml. Cross-reactivity of proinsulin in the radioimmunoassay was evaluated by measuring the profile of C-peptide immunoreactivity (CPR) obtained after gel filtration chromatography of 1 ml plasma samples on 1 × 50-cm Biogel P6 columns equilibrated in 3 M acetic acid. Even after maximal stimulation of C-peptide secretion by intravenous glucose, all the CPR co-eluted with the C-peptide standard and CPR was not detected in the region of the column where the larger proinsulin molecule was found to elute. Thus, under the conditions of these experiments, there was no contribution of proinsulin to total CPR.
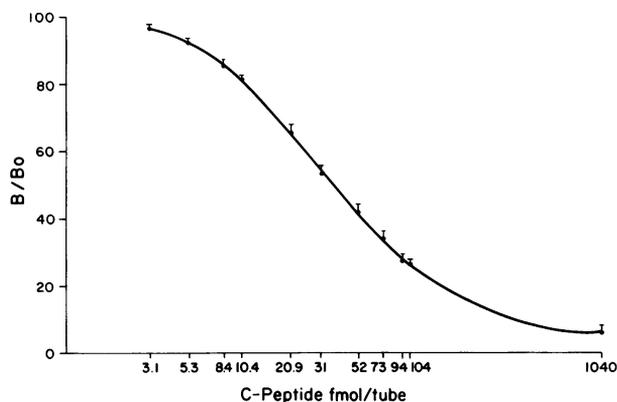
FIGURE 1 Dog C-peptide radioimmunoassay standard curve. Each point represents mean±SEM of values obtained in five successive assays. B/B$_0$, bound/bound in zero dose tube.

In vivo validation of the C-peptide immunoassay was performed by studying the responses of plasma levels of CPR to known stimulatory and suppressive maneuvers. Thus, the fasting arterial C-peptide level increased from 0.20±0.03 to 0.40±0.03 pm/ml ($n = 5$) 45 min after the intravenous injection of 10 g glucose. In six alloxan diabetic dogs, the level of CPR was significantly lower than in control dogs (0.04±0.02 pm/ml) and peripheral CPR was undetectable after total pancreatectomy ($n = 5$), during insulin-induced hypoglycemia ($n = 4$), and during high dose (800 ng/kg per min) somatostatin infusion ($n = 6$).

In vivo experimental dog model. Experiments were conducted in normal conscious mongrel dogs of both sexes weighing 15-25 kg, 17-21 d after placement of sampling catheters in the femoral artery, portal vein, and hepatic vein, as previously described (7). The position of the catheters was verified at autopsy at the end of each experiment.

In the first series of seven experiments, performed after an overnight fast, the hepatic extraction of endogenously secreted C-peptide and insulin was compared with that of exogenously infused C-peptide and insulin. Hepatic plasma flow was calculated from the hepatic clearance of continuously infused indocyanine green (ICG) (17). After a 90-min period during which the indocyanine green was allowed to equilibrate, three base-line samples were drawn at 10-min intervals for C-peptide, insulin, and ICG estimations. A high dose somatostatin infusion (50 μg bolus and 800 ng/kg per min) was then administered. Portal vein and arterial samples were drawn 25 and 30 min after the start of the somatostatin infusion to document complete suppression of insulin and C-peptide levels in both vessels. Thereafter, the hepatic metabolism and metabolic clearance rate of exogeneously infused C-peptide (isolated from dog pancreas as described above) and pork insulin (Regular, Eli Lilly & Co., Indianapolis, IN; identical in structure to dog insulin) was measured during two additional experimental periods.

During each 45 min-period, an appropriate bolus of C-peptide and insulin was administered, followed by a constant intravenous infusion. Samples were drawn from the femoral artery, portal vein, and hepatic vein at 35, 40, and 45 min into the infusion period, once steady state concentrations had been reached. The first C-peptide infusion (50 pm/kg bolus plus 3 pm/kg per min) was calculated to produce peripheral C-peptide levels similar to those found in the fasting state.

The second infusion rate was increased fivefold (250 pm/kg bolus plus 15 pm/kg per min) to mimic stimulated C-peptide levels. After an initial bolus (0.5 U), insulin was infused at constant rate (0.8 mU/kg per min) during both experimental periods. The plasma glucose level was clamped at the basal concentration throughout the experiment by means of a peripheral glucose infusion, the rate of which was adjusted according to the plasma glucose concentration determined at 2-5-min intervals.

In the second series of experiments, the effect of intravenous glucose administration on the hepatic extraction of endogenously secreted C-peptide and insulin was evaluated using the same animal preparation. Again, C-peptide and insulin were sampled from the femoral artery and the portal and hepatic veins and ICG was sampled from the femoral artery and hepatic vein. During a base-line period, four samples were drawn for C-peptide, insulin, and ICG at 15-min intervals. At time 0 the intravenous glucose infusion was begun and samples were obtained every 15 min until 60 min and every 20 min thereafter for a total of 240 min. Intravenous glucose was infused between 0 and 60 min according to three protocols: (a) In five experiments, glucose was administered by peripheral intravenous infusion at a rate that was varied to mimic peripheral levels of glucose previously observed after oral glucose (7). (b) In four experiments, similar peripheral glucose levels were achieved by an intraportal glucose infusion via a mesenteric vein catheter. (c) In four additional experiments, gastrointestinal inhibitory polypeptide (GIP) was administered intraportally (25 ng/kg per min) together with a peripheral glucose infusion as in (a). A mean of 9.8±0.9 g glucose was infused over the duration of the experiment and this amount did not differ in the three groups of dogs.

Sample collection. In all experiments, femoral artery and portal vein samples were drawn simultaneously 25 s before hepatic vein samples to allow for hepatic transit time (9). Blood samples for insulin were allowed to clot at room temperature and the serum 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 data analysis. Serum insulin was measured by a double antibody technique (18). Plasma glucose was measured with a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH).

The hepatic extraction and metabolic clearance rate of C-peptide and insulin, hepatic glucose output, and hepatic plasma flow were calculated as previously described (19, 20).

The significance of differences between group means was evaluated by the two-tailed paired t test or by analysis of variance, where more than two groups were being compared. The analysis of variance model used was a modification of the randomized block design described by Bock (21) for analysis of repeated measurements in the n-sample case. The effects of intravenous glucose administration on the hepatic extraction of C-peptide and insulin in individual dogs was analyzed by linear regression analysis. All results are expressed as mean±SEM and $P < 0.05$ was considered significant. Calculations were performed on an Amdahl 470 V/7 computer (Amdahl Corp., Sunneyvale, CA), with the Statistical Analysis System (SAS Institute Inc., Cary, NC).

RESULTS

The metabolism of C-peptide and insulin under steady state conditions. The results of the seven ex-

periments performed under steady state conditions of C-peptide infusion are shown in Fig. 2 and Table I. Mean values were calculated for each experimental period from the values at each of the three sampling times, since the coefficient of variation of these three values was low (<10%). The mean fasting endogenous levels of C-peptide were 0.24±0.04, 0.39±0.06, and 0.33±0.06 pm/ml in the femoral artery, portal vein, and hepatic vein, respectively (Fig. 2). Somatostatin
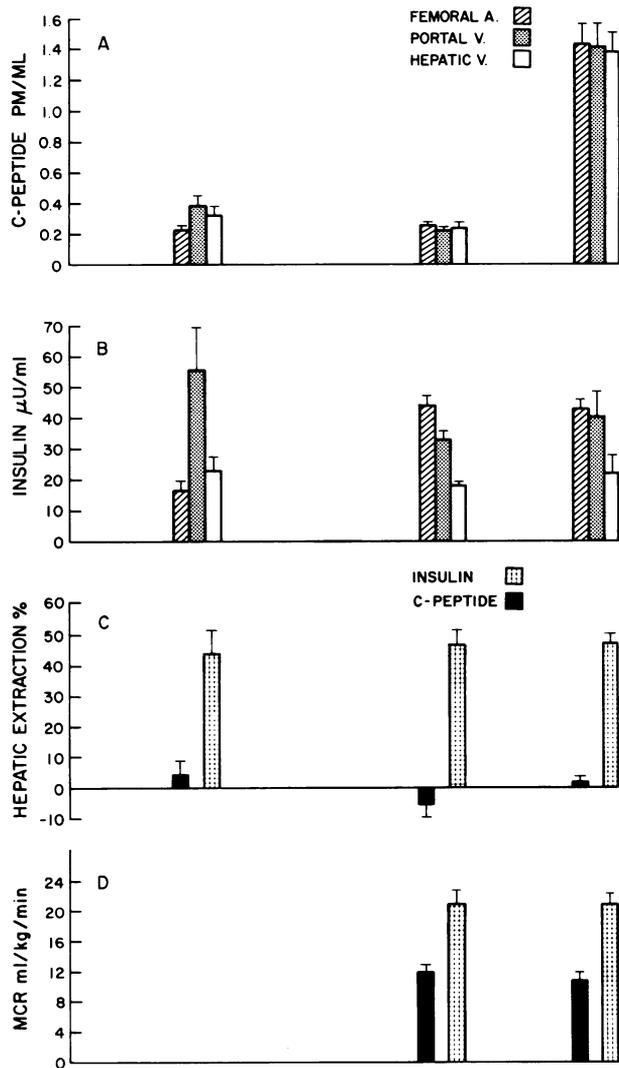

FIGURE 2 The metabolism of C-peptide and insulin in seven dogs under steady state conditions. Simultaneously measured concentrations of C-peptide and insulin are shown in A and B. These concentrations were used to calculate the hepatic extraction and MCR of C-peptide and insulin respectively (C and D). In each of the four panels, data from the endogenous period are on the left, data from the low dose C-peptide infusion are shown in the middle and data from the high dose infusion are on the right.

infusion resulted in suppression of CPR below the detection limit of the assay in all vessels. During the first exogenous infusion period, CPR concentrations of 0.28±0.02, 0.25±0.02, and 0.27±0.03 pm/ml were achieved in the femoral artery, portal vein, and hepatic vein, respectively. During the higher-dose C-peptide infusion, the corresponding C-peptide levels were 1.45±0.14, 1.44±0.16, and 1.40±0.14 pm/ml. In the endogenous period the hepatic extraction of C-peptide was 4.3±4.5% and did not differ from the values of −5.3±4.4 and 2.1±2.4% measured during the low- and high-dose exogenous infusion, respectively, as determined by analysis of variance (Fig. 2). The mean hepatic C-peptide extraction in each dog is shown in Table I.

Concentrations of endogenous insulin were 17±2.6, 56.3±13.4, and 23.2±3.7 μU/ml in the femoral artery portal vein and hepatic vein (Fig. 2). During the two exogenous infusion periods, the arterial levels were 44.8±3.2 and 44.5±2.7 μU/ml and corresponding values in the portal vein were 33.6±2.7 and 41.5±9.5 μU/ml; in the hepatic vein, they were 19.0±1.1 and 23.2±5.9 μU/ml. The hepatic extraction of endogenous insulin (43.8±7.6%) did not differ from that in the two exogenous periods (47.5±4.4 and 47.8±3.8%).

The metabolic clearance rate (MCR) of C-peptide at the two concentrations studied, did not differ by paired t test analysis. The values obtained were 11.9±0.8 ml/kg per min during the low-dose infusion and 11.0±1.2 ml/kg per min during the higher-dose infusion. Since the infusion rate of insulin was constant during both infusion periods, its MCR as expected, also remained constant at 21.1±2.0 and 21.1±1.6 ml/kg per min, respectively.

Hepatic extraction of C-peptide and insulin after intravenous glucose. Having determined that the hepatic extraction of C-peptide was negligible under basal steady state conditions, we next sought to determine whether this also held true after stimulation of C-peptide secretion by parenteral glucose. 13 dogs received parenteral glucose infusions and the data from these experiments are shown in Figs. 3 and 4.

The base-line femoral artery glucose (Fig. 3 A) (121.5±4.8 mg/dl) increased to reach a peak of 188.5±8.1 mg/dl 60 min after the start of the glucose infusion. The levels fell after the glucose infusion was discontinued and returned to basal by 120 min.

Simultaneous changes in hepatic glucose output are shown in Fig. 3 B. The basal hepatic glucose output (2.40±0.41 mg/kg per min) was suppressed rapidly by the glucose infusion and by 60 min a small net hepatic glucose uptake 0.08±0.43 mg/kg per min occurred. After the glucose infusion was discontinued, there was a rapid increase in the rate of hepatic glucose production, which returned to basal levels by 200 min.

TABLE I

Steady State Hepatic Extraction and MCR of C-peptide and Insulin in Seven Dogs

Dog	Hepatic C-peptide extraction	Hepatic insulin extraction	C-peptide MCR	Insulin MCR
	%		ml/kg/min	
1	2.8	48.7	13.4	20.0
2	6.3	38.8	8.5	21.8
3	9.8	—	9.0	—
4	0.2	49.2	12.0	21.6
5	−4.1	51.0	13.2	17.6
6	−11.9	32.0	10.4	17.8
7	−0.7	59.0	13.9	27.8
Mean±SEM	0.3±2.7	46.5±3.9	11.5±0.8	21.1±1.5

Values for hepatic extraction represent the mean of the endogenous and two exogenous infusion periods. The MCR is the mean of the values obtained during the two exogenous infusions.

Fasting C-peptide levels were 0.18±0.02, 0.28±0.03, and 0.24±0.02 pm/ml in the femoral artery, portal vein, and hepatic vein, respectively (Fig. 3 C), and increased approximately fourfold within 60 min after the start of the glucose infusion. These elevated levels returned to base line within 60 min after the glucose infusion was discontinued.

Fasting insulin concentrations were 14.8±2.5, 38.4±5.5, and 15.1±1.4 μU/ml in the femoral artery, portal vein, and hepatic vein, respectively (Fig. 3 D). In response to the glucose stimulus, they increased in parallel to the C-peptide concentrations and returned to base line 60 min after the infusion was discontinued.

The fasting C-peptide/insulin molar ratio (Fig. 4 A) in both the portal vein (1.2±0.1) and femoral artery (2.1±0.3) remained constant throughout the duration of the experiment, as determined by analysis of variance.

In the fasting state, the hepatic extraction of C-peptide was 1.9±4.1% (Fig. 4 B). The simultaneously measured fasting insulin extraction was 46.4±5.1% (Fig. 4 B). To determine whether the hepatic extraction of C-peptide and insulin or their molar ratios in the portal vein and femoral artery varied significantly over the duration of the experiment, an analysis of variance model for repeated measurements in the n-sample case was used (21). This technique allowed possible effects of sampling time on these parameters to be assessed while simultaneously making allowance for individual dog-to-dog variation. In addition, we were able to determine whether the hepatic extraction of insulin and C-peptide or their molar ratios were different in the animals who received peripheral intravenous glucose infusions alone, glucose infusions together with GIP, or intraportal glucose. The results revealed that neither C-peptide nor insulin extraction, or their portal and

peripheral molar ratios were affected by the glucose stimulus, or the co-administration of GIP. The route of glucose administration also had no influence on these parameters.

To determine whether single experiments showed trends not evident from analysis of group mean data, the data from individual animals were analyzed separately by linear regression. For each animal, individual values of C-peptide and insulin extraction were regressed against their respective sampling times. Significant hepatic extraction of C-peptide or insulin was said to exist if the corresponding Y-intercept was significantly greater than zero. A statistically significant slope was taken as evidence of a significant linear change in hepatic extraction in response to the glucose stimulus. Thus, examination of the intercepts and slopes determined for each animal in Table II shows that, although significant hepatic extraction of insulin was present in all the animals, hepatic extraction of C-peptide was significantly greater than zero in only three animals. The calculated estimates of the slopes indicate that hepatic extraction did not change in linear fashion during glucose infusion in the vast majority of dogs. However, one animal demonstrated a statistically significant increase in C-peptide extraction and two animals showed a statistically significant increase in insulin extraction. To exclude a more complex nonlinear effect of glucose infusion on C-peptide and insulin extraction, the residuals of each regression were separately plotted against sampling time for each experiment and examined. Polynomial functions of time to the second power were also fitted. However, no evidence of a consistent change in either C-peptide or insulin extraction following the glucose stimulus was found in the 13 dogs (data not shown).

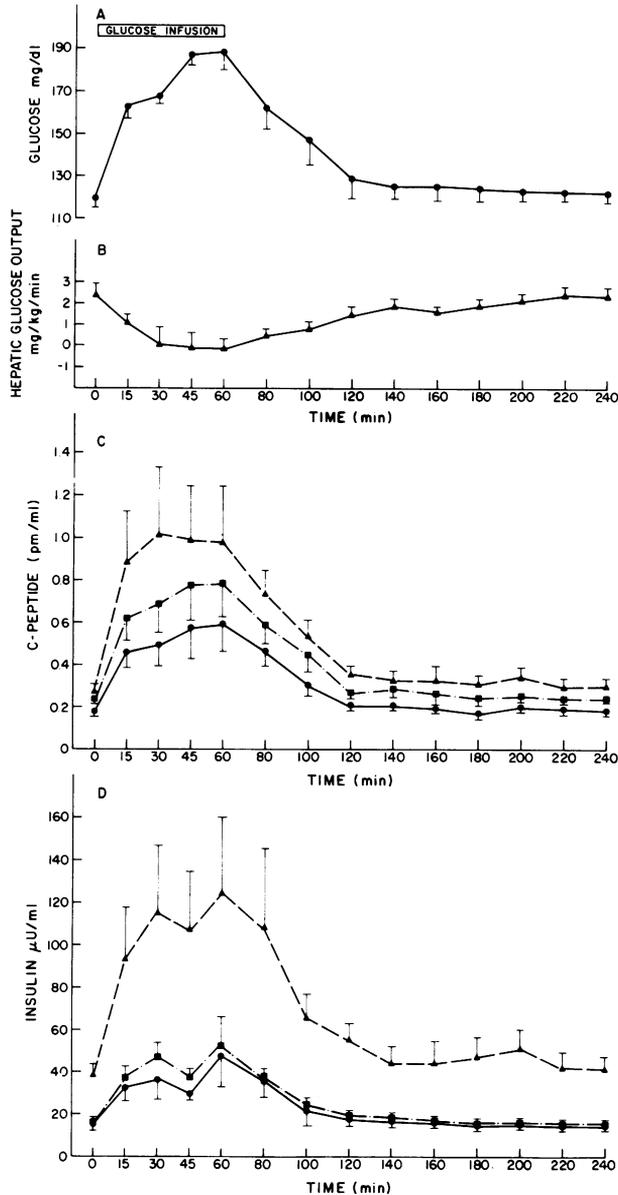To confirm the above observation that route of in-

FIGURE 3 The effect of parenteral glucose infusion in 13 dogs. Glucose was infused between 0 and 60 min. Values at time 0 represent the mean of the values obtained during the base-line period. A and B represent changes in femoral artery glucose and hepatic glucose output respectively. C and D represent simultaneous C-peptide and insulin levels in the femoral artery (●) portal vein (▲) and hepatic vein (■).

travenous glucose administration did not influence hepatic insulin or C-peptide extraction, the slope and intercept parameters were compared in dogs infused with glucose peripherally, intraportally, or peripherally, together with intraportal GIP. By multivariate analysis of variance, the three routes of glucose administration did not differ in their effects on the hepatic extraction of either peptide.

Since hepatic extraction of neither C-peptide nor insulin changed during the glucose infusion, the data for all time points including the base line were pooled to determine overall hepatic extraction in each animal (Table II). It can be seen that the mean hepatic extraction in individual dogs varied from -10.6 to $+35.3\%$ for C-peptide and from 30.1 to 64.2% for insulin with mean values of 6.2±4.0 and 46.7±3.4%, respectively, for the entire group.

DISCUSSION

Insulin and C-peptide are co-secreted from the pancreas on an equimolar basis (11). In the belief that the hepatic extraction of C-peptide is negligible, its peripheral levels have been used as an index of portal insulin and, therefore, beta cell secretory capacity (22–24). In fact, Eaton et al. (25) have calculated the insulin secretion rate in man from changes in peripheral C-peptide kinetics and Waldhausl et al. (8) have used the hepatic vein C-peptide concentration to calculate splanchnic insulin output in human subjects.

The validation of these methods of quantitating insulin secretion depends on a detailed understanding of the hepatic metabolism and peripheral clearance of C-peptide. However, direct experimental data concerning these metabolic processes is incomplete. Although Katz and Rubenstein (26) in the rat and Faber et al. (27) in man have demonstrated that the metabolic clearance rate of C-peptide is linear over a wide concentration range, both studies were conducted only under basal fasting conditions. In addition, little direct data are available concerning the role of the liver in the metabolic disposal of C-peptide. Thus, Stoll et al. (28) reported that the isolated perfused rat liver did not extract significant quantities of bovine C-peptide. Portal peripheral ratios of C-peptide in man as reported by Horwitz et al. (29) were also consistent with negligible hepatic C-peptide extraction. On the other hand, Kuhl et al. (30), in an in vivo study in the pig, reported that the hepatic extraction of C-peptide was 30%. In that study, however, calculations were based on portal and hepatic vein samples only. This technique, which ignores the contribution of the hepatic artery to hepatic C-peptide delivery, would be expected to overestimate hepatic C-peptide extraction. To address these questions directly, a specific and sensitive radioimmunoassay for dog C-peptide was developed. As a result, it has been possible to study the in vivo hepatic and peripheral metabolism of C-peptide for the first time in relation to that of insulin under different physiological conditions.

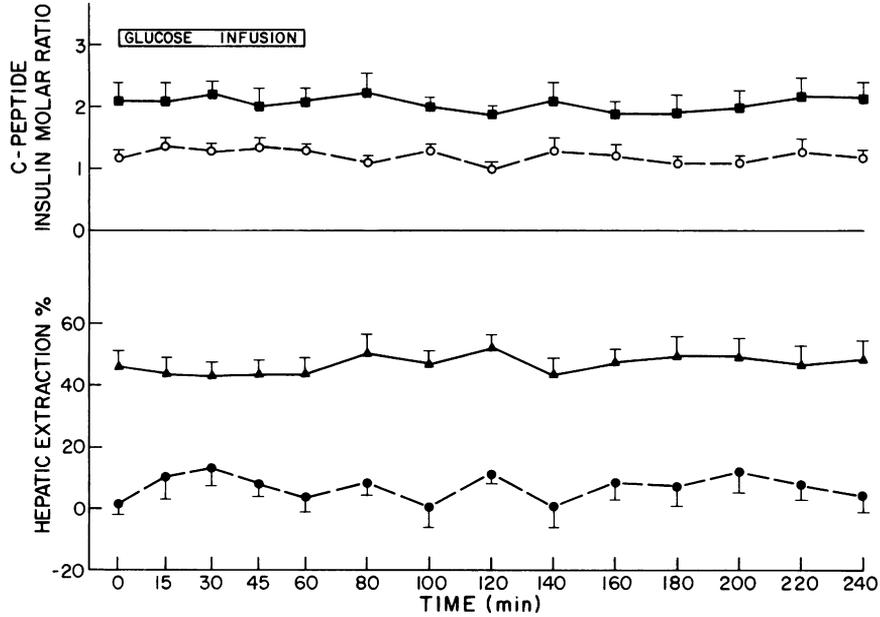In these studies we have been able to establish what

FIGURE 4 The effect of parenteral glucose infusion on the C-peptide; insulin molar ratio in the femoral artery (■) and portal vein (O, upper panel). The simultaneous hepatic extraction of insulin (▲) and C-peptide (●) are also shown in B (lower panel).

has previously been suspected; namely, that the liver is not an important site of C-peptide disposal. In experiments performed under steady state conditions, the overall hepatic extraction of C-peptide was negligible (0.3±2.7%). Neither endogenously secreted C-peptide nor exogenously infused C-peptide (previously

TABLE II
Effect of Glucose Infusion on the Hepatic Extraction of C-peptide and Insulin

	C-peptide					Insulin				
Dog	Intercept	P	Slope	P	Hepatic extraction	Intercept	P	Slope	P	Hepatic extraction
8*	−12.0±5.5	<0.04	0.02±0.04	NS	−10.6±3.9	31.8±4.4	<0.0001	−0.02±0.04	NS	30.1±3.2
9*	35.3±5.5	<0.0001	−0.04±0.04	NS	31.5±3.9	60.4±3.7	<0.0001	−0.01±0.03	NS	59.4±2.6
10*	−0.10±5.1	NS	−0.07±0.04	NS	−6.0±3.6	34.7±7.8	<0.0005	−0.008±0.06	NS	34.0±5.4
11*	26.0±5.5	<0.0003	0.10±0.04	<0.03	35.3±3.9	62.2±3.9	<0.0001	0.02±0.03	NS	64.2±2.8
12‡	−14.3±9.2	NS	0.11±0.07	NS	−5.0±6.5	49.1±7.3	<0.0001	0.07±0.06	NS	55.0±5.2
13‡	6.7±6.4	NS	0.01±0.05	NS	7.6±4.5	55.3±3.9	<0.0001	0.03±0.03	NS	58.2±2.7
14‡	2.0±3.6	NS	0.04±0.03	NS	5.7±2.6	52.1±4.2	<0.0001	−0.02±0.03	NS	50.0±2.9
15‡	−2.0±4.6	NS	0.02±0.04	NS	−0.5±3.2	27.4±4.7	<0.0001	0.05±0.04	NS	32.1±3.3
16‡	15.3±4.0	<0.0015	0.07±0.03	NS	21.1±2.8	51.8±3.3	<0.0001	0.11±0.03	<0.001	61.1±2.3
17§	3.5±3.0	NS	0.02±0.02	NS	5.3±2.1	55.5±4.3	<0.0001	−0.05±0.03	NS	50.8±3.0
18§	3.3±6.4	NS	0.001±0.05	NS	3.4±4.5	25.8±3.4	<0.0001	0.09±0.03	<0.01	33.5±2.4
19§	2.9±3.3	NS	−0.04±0.03	NS	−0.6±2.3	45.5±5.2	<0.0001	−0.04±0.04	NS	41.8±3.6
20§	−6.2±4.1	NS	−0.001±0.03	NS	−6.3±2.8	36.7±5.4	<0.0001	0.01±0.04	NS	37.3±3.8
Mean±SEM	4.6±3.9	NS	0.018±0.01	NS	6.2±4.0	45.3±3.5	<0.0001	0.02±0.01	NS	46.7±3.4

The slope and intercept parameters were derived by regression of hepatic extraction of C-peptide and insulin against experimental sampling times. Hepatic extraction is the mean value in each experiment. Data are expressed as mean±SEM. P values refer to the confidence with which the null hypothesis that slope and intercept were zero could be rejected.
* Infusion of glucose and GIP.
‡ Infusion of glucose intravenously.
§ Intraportal glucose infusion.

isolated from the dog pancreas) was extracted by the dog liver to any significant extent. Simultaneous measurement of the hepatic extraction of insulin (46.5±3.9%) was similar to values reported by other workers (1, 2, 9). This demonstrates the validity of our experimental model and confirms the accuracy of the catheter placement and hormone sampling techniques.

The effect of intravenous glucose infusion on the hepatic extraction of insulin and C-peptide was also studied. Glucose was administered by peripheral infusion, alone or together with GIP or by intraportal infusion. In these animals, the base-line hepatic extraction of C-peptide was also not significantly different from zero (1.9±4.1%) and did not change after glucose infusion, irrespective of the route by which the glucose was administered. Hepatic insulin extraction was also not affected by glucose stimulation and remained between 40 and 55% throughout the experiment.

In the present experiments, we have also been able to demonstrate that the metabolic clearance rate of C-peptide is not altered when its concentration is raised from 0.28±0.02 to 1.45±0.14 pm/ml by intravenous infusion. This observation, which is an important aspect of the demonstration that C-peptide is a valid indicator of insulin secretion over the range of concentrations encountered under physiological circumstances, has now been made in three species; namely, dog, rat (26), and man (27). Differences between the metabolism of C-peptide in the dog and other species do exist however. Thus, the peripheral C-peptide/insulin molar ratio (2.1±0.3) in the dog is consistent with the ratio of insulin MCR to C-peptide MCR (1.8±0.2) in that species. In man, however, the MCR of C-peptide is much slower relative to that of insulin (27), resulting in a peripheral C-peptide/insulin molar ratio of ~10:1 in the fasting state (29, 31). These differences are probably related to differences in the amino acid composition and size of the C-peptide molecule in the two species. In man, C-peptide is a 31-amino-acid peptide, whereas in the dog it is considerably smaller, consisting of only 23 amino acids (12). It is likely that the renal metabolism of this smaller peptide is greater in the dog, thus accounting for its more rapid metabolic clearance rate and lower peripheral levels.

Accurate quantitation of the true magnitude of hepatic C-peptide extraction and its variability in individual dogs is of considerable importance. The pattern of hepatic metabolism of C-peptide and insulin was therefore analyzed separately in individual dogs as shown in Table II. Highly significant hepatic insulin extraction was documented in each dog (range 30–64%). In contrast, in nine of the 13 animals, the mean hepatic C-peptide extraction was not statistically different from zero. Three of the remaining animals, however, demonstrated consistent hepatic C-peptide extraction (31.5±3.9, 35.3±3.9, 21.1±2.8%) and, in the fourth, the measured extraction was consistently negative. The reason for these differences in individual animals is uncertain. It is interesting to note, however, that the dogs who demonstrated hepatic metabolism of C-peptide also showed hepatic insulin extraction (59.4, 64.2, and 61.1%) that was greater than the other animals. It is possible that in these animals, the liver had a greater propensity for the metabolism of small peptides and thus extracted C-peptide as suggested by Kuhl et al. (30). However, the possibility that the apparent hepatic C-peptide extraction was an experimental artifact must also be considered. A consistent sampling error is the most likely source of such an error. Since the sampling catheters were in the correct anatomic position at autopsy performed at the completion of each experiment, the sampling error would have had to result from streaming effects in the portal and hepatic veins as described by Kanazawa et al. (32). Although such effects are difficult to exclude with certainty, it should be noted that in each of these animals the femoral artery C-peptide concentrations were lower than the corresponding hepatic venous concentrations. Streaming can definitely be said to have occurred, if the arterial concentration is greater than the hepatic venous concentration of endogenously secreted peptide. The present data indicate that, although in the vast majority of dogs the hepatic extraction of C-peptide is not significantly different from zero, it appears possible that in a small proportion of animals the liver does extract C-peptide. A sampling error is the most likely source of the consistently negative extraction observed in dog 8.

An understanding of the factors that regulate the metabolism of insulin is of considerable importance to our understanding of insulin kinetics (1–9, 20, 25, 28, 29, 31–33). We previously noted a marked but transient increase in hepatic insulin extraction in dogs after glucose ingestion (7). Others have made similar observations (2), although it has also been suggested that feeding may reduce hepatic insulin extraction (33). We have now demonstrated that intravenous glucose infused at a rate that results in peripheral glucose levels similar to those found after oral glucose does not alter the hepatic extraction of either insulin or C-peptide. Despite an approximate fourfold increase in hepatic insulin and C-peptide delivery after the glucose stimulus, the hepatic extraction of both peptides remained constant. These results indicate that the increase in insulin extraction seen after oral glucose is not due to the increase in glucose levels per se or to the increase in hepatic insulin delivery resulting from

the glucose stimulus. Although it has been suggested that GIP increases insulin secretion after oral glucose, it does not appear to regulate hepatic insulin extraction. The differences between the effects of oral and intravenous glucose suggest that some other presumably gut factor, yet to be identified, can modify hepatic insulin extraction.

In summary, the present data provide direct evidence that in the dog changes in peripheral C-peptide levels accurately reflect changes in portal insulin concentrations and therefore rates of insulin secretion both under basal fasting conditions and after stimulation by intravenous glucose. It still remains to be shown that the relationships between C-peptide and insulin metabolism demonstrated in the present study pertain to other physiological situations, such as after oral feeding. Nevertheless, exciting new possibilities for the quantitation of hepatic extraction and secretion of insulin by noninvasive techniques are now feasible.

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