Kinetics of Human Connecting Peptide in Normal and Diabetic Subjects

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ABSTRACT The metabolic clearance rate (MCR) of synthetic human connecting peptide (C-peptide) was measured with a single-dose injection technique in six normal and seven diabetic subjects and with a constant infusion technique in one normal subject.

The MCR of C-peptide did not differ in normal subjects (4.4 ml/min per kg; range, 3.7–4.9) and in diabetic subjects (4.7 ml/min per kg; range, 3.7–5.8). Employment of both techniques in one subject gave similar MCR. The average half-life of C-peptide in plasma calculated from the last 1-h period of the single-dose injection studies was longer in the insulin-dependent diabetics (42.5 min; range, 39.4–48.5) than in the normal subjects (33.5 min; range, 24.9–45.3).

These results indicate that the β-cell secretory capacity of normal and insulin-dependent diabetic subjects can be compared by measuring the C-peptide concentration in peripheral venous plasma. The difference in the half-life of C-peptide in plasma between diabetics and normals suggests an altered kinetics of the disappearance of the peptide, while the overall metabolism, as expressed by the MCR, is similar.

INTRODUCTION

Our knowledge of the kinetics of insulin is usually derived from measurements of peripheral blood concentrations of the hormone after its infusion into the systemic circulation. Owing to the varying and substantial uptake and degradation of insulin in the liver (1–3), such measurements do not form an adequate basis for the quantitation of pancreatic insulin secretion.

Insulin is produced in the pancreatic β-cells by cleavage of its single-chain polypeptide precursor, proinsulin, into insulin, the connecting peptide (C-peptide),[1] and two pairs of basic amino acids (4, 5). Insulin and C-peptide are subsequently secreted into portal circulation in equimolar quantities (6–8) along with small amounts of proinsulin and its intermediate forms (9–11). In contrast to insulin, C-peptide is not extracted from the circulation to any significant degree by the liver (12).

For this reason, the determination of the C-peptide concentration in peripheral venous plasma yields an indirect, but more accurate measure of the insulin secretory activity of the pancreatic β-cells (13, 14, 7, 8). Provided that the kinetics of the removal of C-peptide from the circulation are known, the insulin secretion rate can be quantitated from peripheral C-peptide concentrations. This also applies to insulin-treated diabetics in whom exogenous insulin as well as endogenous insulin antibodies interfere with the measurement of endogenous insulin (13).

In animals (15–17) as well as in humans (18, 19), the turn-over of C-peptide is slower than that of insulin. However, detailed studies concerning the kinetics of C-peptide turnover have not been carried out in normal man. The purpose of this study was to determine these values as well as to examine whether

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[1] *Abbreviations used in this paper*: C-peptide, connecting peptide; MCR, metabolic clearance rate.

TABLE I

Individual Clinical Data and Calculated Kinetic Parameters from Single-Dose Injection Studies of Human C-Peptide in Normal and Diabetic Men

Subject	Age	Weight	Height	DM duration	Insulin dose	Fasting C-peptide	Fasting insulin	Dose of C-peptide	MCR	t½
	yr	*kg*	*cm*	*mo*	*U/24 h*	*pmol/ml*	*pmol/ml*	*pmol*	*ml/min*	*min*
C. B.	43	86	181	—	—	0.14	0.06	29,420	315	45.3
O. F.	33	71	180	—	—	0.15	0.04	24,000	340	24.9
C. Ha.	32	61	180	—	—	0.28	0.07	34,500	296	37.3
C. He.	27	63	178	—	—	0.31	0.04	35,270	274	34.1
S. M.	31	80	192	—	—	0.20	0.07	31,870	317	27.8
T. L.	25	75	187	—	—	0.35	0.07	32,530	319	31.4
Mean	32	73	183	—	—	0.24	0.06	31,265	310	33.5
Range	25–43	61–86	180–192	—	—	0.14–0.35	0.04–0.07	24,000–35,270	296–340	24.9–45.3
P. N.	38	70	181	14	36	0.05	—	33,930	314	46.2
B. A.	26	73	177	14	20	0.12	—	32,420	384	39.8
P. L.	48	62	172	18	16	0.08	—	35,860	325	40.5
O. N.	30	70	171	13	24	0.05	—	33,590	295	42.0
B. P.	44	68	182	13	24	0.24	—	32,450	392	39.4
G. L.	30	66	182	19	40	<0.02	—	30,290	284	41.0
M. C.	38	72	182	22	28	0.08	—	26,660	266	48.5
Mean	36	69	178	16	27	0.09	—	32,170	323	42.5
Range	26–48	62–72	171–182	13–22	16–40	<0.02–0.24	—	2,660–35,860	266–384	39.4–48.5
P	NS	NS	NS	—	—	<0.02	—	NS	NS	<0.05

The *P* values are calculated from Mann-Whitney's rank sum test for unpaired data.
DM, diabetes mellitus.

the C-peptide metabolism differs in normal subjects and diabetic patients.

METHODS

Preparation of synthetic human C-peptide solutions. A stock solution of synthetic human C-peptide (20) was made by dissolving 20 mg freeze-dried C-peptide in 14 ml distilled water. The concentration of the stock solution was determined to be 460 nmol/ml by amino acid analysis (21) by using the JEOL JLC-5AH amino acid analyzer (JEOL Analytical Instruments, Cranford, N. J.). 2 ml of the stock solution and 8 ml 0.12 M sodium chloride were sterilized by filtration through a Millipore filter (Millex 0.22 μm, Millipore Corp., Bedford, Mass.). The concentration of this solution was 92 nmol/ml, the loss due to adsorption onto glassware being negligible.[2]

For the single-injection studies, ≅0.35 ml of the sterile solution was added to 10 ml of isotonic, sterile sodium chloride within 12 h before the actual study. In the case of the constant-infusion study, the respective quantities were ≅3.1 and 90 ml. The dose of C-peptide injected was calculated from the volume of C-peptide, measured by weight, and the concentration of C-peptide, measured by immunoassay. The concentration of the injected sterile C-peptide solution measured by immunoassay agreed within 100.1±5.6% (mean±1 SD) with that estimated, after dilution of the original solution.

Single-dose injection. The plasma C-peptide concentration, after a bolus injection of unlabeled C-peptide, was determined in six normal subjects and seven insulin-dependent diabetic men (Table I). Studies were performed on recumbent subjects who fasted overnight (12–14 h). An intravenous catheter was placed in a cubital vein and kept patent with a slow infusion of sodium chloride. After a 30-min rest, blood samples were taken at 10-min intervals for 60 min. Thereafter, a known amount of human C-peptide was injected intravenously over 10 s into the contralateral arm. During the subsequent 120 min, 32 blood samples were taken at 1-min intervals for the first 10 min, at 3-min intervals from 12 to 30 min, at 5-min intervals from 30 to 90 min, and then at 10-min intervals.

As described for ^{131}I-insulin (22), the metabolic clearance rate (MCR) for C-peptide was calculated from the plasma C-peptide curves by a stochastic, noncompartmental analysis employing the principle enunciated by Meier and Zierler (23). According to this approach, MCR is expressed as the injected amount of C-peptide (measured in picomoles) divided by the area under the plasma curve from time zero to infinity (picomoles per milliliter multiplied by minutes). The MCR is thus expressed in milliliters per minute and, when related to the body weight, in milliliters per minute per kilogram.

The mathematical analysis was performed as described for albumin (24) and transcobalamin (25). The final rate constant of the plasma disappearance curve was constructed by regression analysis of the exponential curve from 60 to 120 min (in subjects C. B. and O. F. from 30 to 60 min). The rate constant (k) was used for calculation of the half-life of C-peptide ($t_{1/2}$).

The plasma curve $P(t)$ was further resolved into exponential components by graphic interpolation:

$$P(t) = c_1 \cdot e^{-k_1 t} + c_2 \cdot e^{-k_2 t} + c_3 \cdot e^{-k_3 t},$$

where t is the time in minutes, k denotes the rate constant of

[2]Markussen, J. Unpublished observation.

the individual exponential component characterizing $P(t)$, and c is the intercept with the logarithmic ordinate of the individual exponential component of $P(t)$. Integration of this equation qives

$$P(t) = \sum_{i=1}^{i=3} \frac{c_i}{b_i}.$$

Constant infusion. MCR was defined as the volume of plasma cleared of C-peptide in a unit of time. Because of limitations in the amount of C-peptide available, MCR was determined in only one healthy man (C. B.) by use of the constant-infusion technique described for unlabeled human insulin (26).

After an overnight fast, catheters were placed intravenously in both forearms for infusion and blood sampling, respectively. After a 30-min rest, basal-blood samples were taken at 10-min intervals for 60 min. C-peptide was infused by a Braun precision pump (Unita, B. Braun Instruments, San Francisco, Calif.) at three rates during successive 60-min periods. The infusion rates were 0.200, 0.375, and 0.750 ml/min, respectively. At the start of each infusion period, a bolus injection consisting of 6–8% of the total dose of C-peptide for the subsequent infusion period was given. Blood samples were taken at 10-min intervals during the three infusion periods and at rapid intervals in the 120 min after the end of the last period (Fig. 1).

The infused amount of C-peptide per minute was calculated from the infusion rate and the concentration of C-peptide in the infusate. MCR was calculated from the formula (26): MCR = (infusion rate of human C peptide)/(concentration of C peptide in plasma), expressed in milliliters per minute, the plasma C-peptide concentration being the infusion-dependent steady-state concentration obtained by subtracting the fasting C-peptide concentration from the mean steady-state concentration measured during the last 20 min of each infusion period. When related to the body weight, MCR was expressed in milliliters per minute per kilogram. The k of the postinfusion C-peptide disappearance curve was calculated from an exponential curve fit obtained with the method of the least squares. This assumes a first-order system. The half-life of C-peptide in plasma ($t_{1/2}$) is related to k with the formula: $t_{1/2} = 0.693/k$ (27).

Gel filtration of serum and urine. A urine sample collected during the 180—235-min period of the constant-infusion study (Fig. 1), and a serum sample taken at 240 min in the same study, were gel filtered on a 2.0 × 60-cm column of Bio-Gel P-30 (100–200 mesh) (Gel filtration Materials, Bio-Rad Laboratories, Richmond, Calif.) equilibrated in 3 M acetic acid. The column was calibrated with 0.3 ml 20 ng/ml human C-peptide and 0.1 ml 200 ng/ml human proinsulin dissolved in 3 M acetic acid. 1.5-ml urine and 0.8-ml serum samples were brought to a 2.5-ml vol with 3 M acetic acid. The 3.0-ml fractions were dried overnight in a desiccator using sodium hydroxide, and reconstituted in 1.0 ml of the radioimmunoassay buffer (borate-albumin, pH 8.0). Aliquots of each fraction were measured in the C-peptide assay. The percent recovery was as follows: percent recovery = 100 × total C-peptide immunoreactivity in column fractions ÷ C-peptide immunoreactivity in the serum or urine sample.

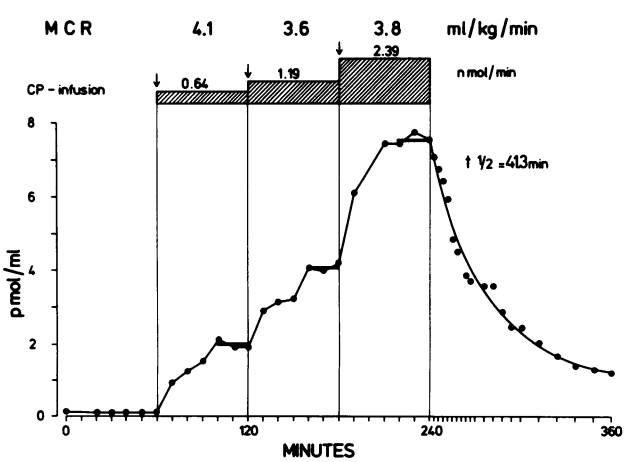

FIGURE 1 The plasma C-peptide concentration before, during, and after constant infusion of synthetic human C-peptide at three different rates in subject C. B.

Chemical and immunological methods. The blood samples were taken in plastic tubes to which aprotinine (450 KIE/ml blood,Trasylol Bayer, FBA Pharmaceuticals, N. Y.) and heparin (45 μU/ml blood, Novo Research Institute, Copenhagen, Denmark) were added. After centrifugation, the plasma was pipetted into plastic tubes and, together with a sample of the infusate, stored at −20°C until assayed. All analyses were made within 50 days after sampling.

The C-peptide concentration in the infusate and plasma samples was determined using antiserum M 1230 (28) as described by Heding (29). All samples from each study were analyzed in the same assay. The intraassay variation coefficient was 3.2% and the detection limit was 0.02 pmol/ml of C-peptide (28).

In the gel-filtration experiments, C-peptide immunoreactivity was determined in fraction aliquots in two C-peptide assays as described by Kuzuya et al. (30) using antisera Y (30) and M 1230 (28).

The plasma-insulin concentration was measured as described by Heding (31).

Statistical methods. The results were analyzed using the Mann-Whitney rank sum test for unpaired data. The significance of correlations were tested by means of Spearman's rank correlation test. 5% was accepted as the lowest level of statistical significance.

RESULTS

Single-dose injection (Table I, Fig. 2). The plasma-insulin concentration did not vary systematically during the period of study in any of the nondiabetic men.

The C-peptide curves could all be resolved into three exponential components. Utilizing these components, the area under the disappearance curve was calculated from time zero to infinity.

The MCR did not differ significantly between normal and diabetic men, neither when expressed in milliliters per minute (Table I), nor when expressed in relation to body weight. In the latter case, MCR averaged 4.4 ml/min per kg (range, 3.7–4.9) in the nondiabetics and 4.7 ml/min per kg (range, 3.7–5.8) in the diabetics.

All the disappearance curves reached final exponential decline with r values for the exponential curve fits ranging from 0.9367 to 0.9939 (mean, 0.9661) in the normal subjects, and from 0.9027 to 0.9958 (mean, 0.9733) in the diabetics. The mean

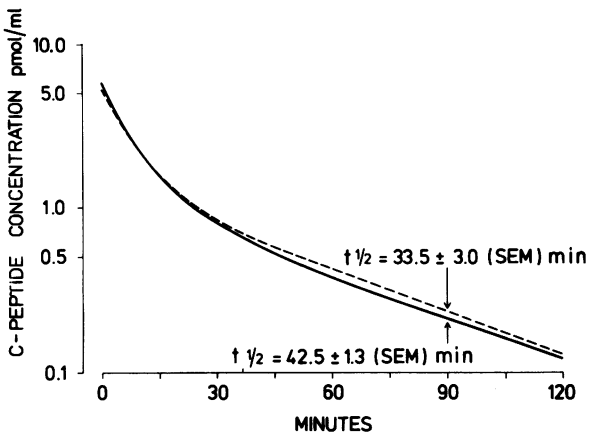

FIGURE 2 The average plasma C-peptide concentration (semilogarithmic scale) in six normal (----) and in seven diabetic (——) men after i.v. injection of synthetic human C-peptide. The half-life for C-peptide in plasma is given for either group.

half-life for plasma C-peptide, calculated from this final slope, was shorter in the nondiabetics (mean, 33.5 min) than in the diabetics (mean, 42.5 min) ($P < 0.05$). In the diabetics, MCR and half-life were inversely correlated ($r = -0.89$, $P < 0.05$, Spearman's rank correlation coefficient), but these two parameters failed to correlate in the nondiabetics or in the group taken as a whole.

Constant infusion (Fig. 1). After the infusion of 0.64, 1.19, and 2.39 nmol/min C-peptide, the steady-state concentrations (mean±SD) during the last 20 min of each infusion period were 1.99±0.11, 4.05±0.10, and 7.54±0.14 pmol/ml, respectively. The plasma-insulin concentrations showed no systematic changes and varied between 0.03 and 0.08 pmol/ml, with an average of 0.06 pmol/ml. Average fasting C-peptide concentration was 0.20 pmol/ml.

MCR during the three infusion periods was 355, 309, and 325 ml/min, respectively (mean±SD = 330 ±23). Expressed in relation to the body weight, MCR averaged 3.8±0.3 ml/min per kg. Variations in the steady-state C-peptide concentration did not influence the MCR. The postinfusion curve for plasma C-peptide had a monoexponential course ($r = 0.9858$) with $k = 0.0168$ per min, corresponding to a $t_{1/2} = 41.3$ min.

Gel filtration of serum and urine (Fig. 3). The C-peptide peaks from the urine and plasma samples eluted coincidentally with that of the C-peptide standard. The position of the peak was the same with both antibodies. The recovery in the serum sample was 71.3% when measured with antiserum M 1230, and 82.3% when measured with antiserum Y. The corresponding values for the urine sample were 86.0 and 73.7%.

DISCUSSION

The development of an immunoassay for human C-peptide has provided an alternative means of studying the β-cell secretory capacity in insulin-treated patients, in whom exogenous insulin and circulating antibodies to insulin interfere with the quantitative measurement of endogenous insulin (13, 29). C-peptide and insulin are secreted in equimolar quantities (6–8), and C-peptide is not extracted to any significant degree by the liver (12). Consequently, the simultaneous determination of C-peptide and insulin in peripheral venous plasma has been used as a relative measure of hepatic insulin uptake (32, 33), and C-peptide measurements alone provide an adequate means for assessing the insulin secretion. However, this latter approach has been limited by our incomplete knowledge of the kinetics of C-peptide. The molar ratio of C-peptide and insulin is approximately 5 in normal fasting individuals and between 2 and 3 after β-cell stimulation (34). This finding suggests that the disappearance of C-peptide is slower than that of insulin. Experiments in animals have corroborated this hypothesis. Thus, in rats infused with bovine insulin, proinsulin, and C-peptide, Katz and Rubenstein (15) found the MCR for insulin to be three to four times faster than that for C-peptide.

Studies in man have also shown a slower clearance of C-peptide as compared to insulin. Horwitz et al. (18) followed the C-peptide concentration in two pa-

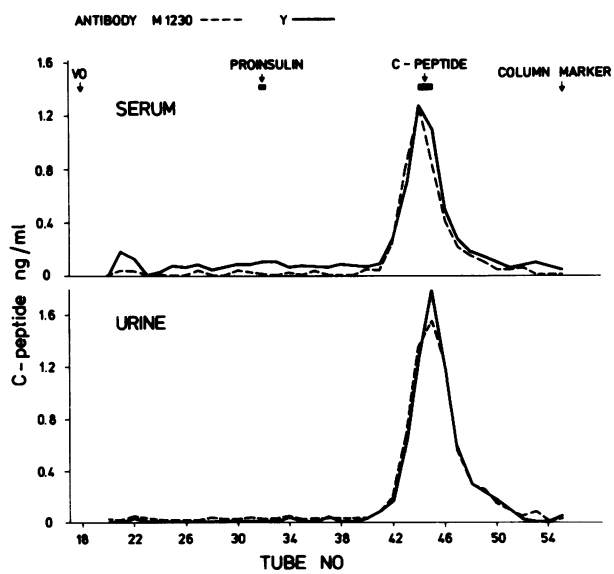

FIGURE 3 The gel-filtration pattern of serum and urine from subject C. B. on Bio-Gel P-30 eluted with 3 M acetic acid and measured with antisera Y (——) and M 1230 (----). Elution positions of natural human proinsulin and C-peptide are indicated by the horizontal bars.

tients after the removal of insulinomas and found the mean $t_{1/2}$ = 11.1 min. Kuzuya and Matsuda (19) determined the endogenous serum C-peptide concentration in six patients after the discontinuation of an infusion of glucose and glucagon, and found the $t_{1/2}$ to average 20.1 min. These values are approximately twice as high as the half-life reported for insulin (35, 36, 19). However, these two values for the half-life of C-peptide are considerably different and are lower than our mean $t_{1/2}$ of 33.5 min.

Cross-reaction of human proinsulin in the C-peptide assay might explain the shorter half-life reported for endogenous C-peptide (18, 19) (the half-life for proinsulin (37–39, 26) being shorter than the half-life for C-peptide obtained in the present study). The influence of human proinsulin will be especially marked in patients with insulinomas because of their raised proinsulin concentrations (39). An additional explanation for the discrepancy might be that synthetic rather than natural human C-peptide was used in the present study. Nevertheless, the gel-filtration pattern of serum and urine samples, obtained during the constant infusion at the time of the highest C-peptide concentration, showed that the synthetic material and endogenous human C-peptide behaved similarly with respect to their elution positions, dilution slopes, and recovery. Moreover, consistent results were obtained with the two C-peptide antisera, each of which reacts with a different region of the C-peptide molecule. These results do not exclude the possibility that the discrepancy is due to a difference in the degradation of synthetic and endogenous human C-peptide, but there is little evidence to support this conclusion. The resolution of this issue must await additional studies with natural and other preparations of synthetic C-peptide.

The MCR for C-peptide, calculated on the basis of a stochastic, noncompartmental analysis of the disappearance curve after a bolus injection of synthetic human C-peptide, averaged 4.4 ml/min per kg in normal men. This value is similar to the MCR for porcine proinsulin in man (26) and is consistent with the results of Katz and Rubenstein (15) who showed that the MCR of proinsulin and C-peptide are similar in the rat.

If one accepts that C-peptide is not extracted to any significant extent by the liver (12) before it reaches the general circulation, it is possible to calculate the basal-insulin secretion when the MCR of C-peptide is known and the fasting C-peptide concentration is taken as an expression of the basal insulin secretion. In the normal subjects, the average fasting C-peptide concentration of 0.24 pmol/ml and MCR concentration of 310 ml/min indicates a basal C-peptide secretion of 107 nmol/24 h equal to 15.5 U of insulin. This is consistent with the basal insulin delivery rate into the systemic circulation in normal subjects reported by Turner et al. (40).

A condition for the mathematical analysis of the plasma C-peptide curve is that the relative rate of degradation is independent of the C-peptide concentration. As the MCR for C-peptide did not differ at the three C-peptide concentrations in the constant-infusion study, a significant concentration dependency seems improbable. Such a dependency has been demonstrated for insulin and, to a lesser degree, proinsulin in normal man (26). It has been suggested that the decreasing fractional clearance rate found with increasing concentration is caused by a saturable hepatic extraction of these peptides (26). The hepatic extraction of the hormones is probably related to their metabolic effects (41). It is therefore to be expected that the overall clearance of C-peptide is independent of its concentration because C-peptide is not cleared by the liver to any significant extent (12) and because it has no metabolic effects (42). Finally, animal studies indicate that the MCR for C-peptide is independent of its plasma level (15). Another condition is that the curve reaches its final slope within the investigation period. This was apparently the case, because the individual k values did not change significantly after 90 min.

The close agreement between the constant infusion and single-dose injection results in the healthy subject in whom both types of study were carried out also suggests that the mathematical approach used was appropriate for the plasma C-peptide curves obtained after single-dose injections. Thus, the MCR calculated from the constant infusion was 3.8 compared to 3.7 ml/min per kg after the single-dose injection. The corresponding values for $t_{1/2}$ were 41.3 and 45.3 min, respectively.

The MCR for C-peptide did not differ significantly in the nondiabetic and the diabetic subjects. This means that the β-cell secretory capacity of normal and insulin-dependent diabetic subjects can be compared by measuring the C-peptide concentration in peripheral venous plasma. However, the kinetics of C-peptide differ in the two conditions, the mean half-life for C-peptide being 27% longer in the diabetic men. We do not know the explanation for this paradoxical finding of a prolonged half-life together with a small, but statistically insignificant increase in MCR in the diabetics. It is of interest that a similar discrepancy has been found for glucagon (43). The most likely explanation is that the half-life is prolonged because of an increase in the distribution space of C-peptide in the diabetic subjects. This would lead to an alteration in the kinetics of the disappearance of the pep-

tide, while the overall metabolism, as expressed by MCR, would remain unchanged.

Addendum. The experimental values obtained for plasma C-peptide concentration (picomoles per milliliter) before (−60–0 min) and after (1–120 min) intravenous injection of synthetic human C-peptide in six normal and seven diabetic subjects have been deposited in table form in the National Auxiliary Publication Service.[3]

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[3] An Appendix has been deposited with the National Auxiliary Publications Service (NAPS) as NAPS document 03235. This information may be ordered from ASIS/NAPS, Microfiche Publications, P. O. Box 3513 Grand Central Station, New York 10017. Remit in advance $3.00 for microfiche copy, or for photocopy, $5.00 up to 20 pages plus 25¢ for additional pages. Checks should be made payable to Microfiche Publications.

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