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A. Tura, G. Pacini, A. Kautzky-Willer, B. Ludvik, R. Prager and K. Thomaseth
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C. B. Juhl, N. Porksen, J. Sturis, A. P. Hansen, J. D. Veldhuis, S. Pincus, M. Fineman and O. Schmitz

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Distribution and kinetics of amylin in humans

M. CLODI,¹ K. THOMASETH,² G. PACINI,² K. HERMANN,¹ A. KAUTZKY-WILLER,¹
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Clodi, M., K. Thomaseth, G. Pacini, K. Hermann, A. Kautzky-Willer, W. Waldhäusl, R. Prager, and B. Ludvik. Distribution and kinetics of amylin in humans. *Am. J. Physiol.* 274 (Endocrinol. Metab. 37): E903–E908, 1998.—The aim of the study was to determine the apparent volume of distribution (V_{TOT}), total body clearance (CL), fractional clearance, and mean residence time (MRT) of the β -cell hormone amylin. We therefore performed an intravenous injection of 50 μg of human synthetic amylin (amlintide) in nine healthy male subjects during suppression of endogenous amylin release by intravenous somatostatin (0.06 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). The plasma levels of amylin concentrations over time were analyzed using three-exponential curves. V_{TOT} was 173 ± 16 ml/kg and was not different from that of insulin reported in the literature (157 ml/kg). MRT was 27.7 ± 2.1 min and thus two times the reported value for insulin (14.1 min) and C-peptide (16.4 min). CL and fractional CL were 6.2 ± 0.2 ml $\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and 0.038 ± 0.003 min $^{-1}$, respectively. Fractional CL is therefore definitely lower than that reported for insulin (0.12–0.2 min $^{-1}$) but is, however, in the range of that of C-peptide (0.05 min $^{-1}$). In conclusion, clearance of amylin is similar to that reported for C-peptide and much slower than insulin, indicating that the commonly used molar insulin-to-amylin ratio does not reflect the correct relationship of the two peptides.

pharmacokinetics; noncompartmental analysis; compartmental analysis; mathematical modeling

IN 1987, pancreatic amyloid was shown to be composed primarily of aggregates of amylin or islet amyloid polypeptide, a peptide containing 37 amino acid residues (8, 34). The chemical structure is nearly 50% homologous with that of calcitonin gene-related peptide (CGRP), which has the same number of amino acid residues and is a widespread neurotransmitter with many potent biological actions (34). In *in vitro* and in animal experiments, amylin has been shown to cause dose-dependent increases in lactate and blood glucose levels (38) and to impair glycogen synthesis through dose-dependent inhibition of insulin-stimulated incorporation of glucose into glycogen in muscle (6, 19). Amylin, which increases glycogenolysis (37) and stimulates hepatic gluconeogenesis from lactate (4), might act as a noncompetitive inhibitor of insulin (37). These actions, however, have been obtained *in vitro* or *in vivo* in animals only by administration of pharmacological doses. In human subjects, however, only a high-dose infusion of amylin decreased insulin secretion (3), whereas insulin action was not changed (35). These findings did not suggest an effect of circulating amylin on glucose metabolism in humans.

Amylin is secreted by pancreatic β -cells in response to nutrient stimuli together with insulin (12). In obese

subjects, amylin is increased in parallel with insulin in the presence of insulin resistance (22). In type 1 diabetes amylin secretion is absent (11), whereas in type 2 diabetes its secretion is impaired before that of insulin (22). Recently, it has been shown that administration of an amylin antagonist led to an increase of insulin secretion (18), suggesting that amylin might inhibit insulin secretion under physiological conditions. Furthermore, the amylin agonist pramlintide (tri-pro amylin) is able to decrease postprandial glucagon secretion in type 1 diabetic subjects (27), thereby reducing continuing postprandial hepatic glucose output and thus hyperglycemia. The administration of pramlintide has been shown to restore accelerated gastric emptying in patients with diabetes (16) and thus improve postprandial metabolic control (15, 26). Based on these findings, amylin seems to act as a pancreatic hormone in controlling glucose metabolism.

Relatively little, however, is known with regard to the metabolism of amylin in humans. In a previous study, glucose, insulin, C-peptide, and amylin serum levels have been measured in insulin-resistant subjects and in healthy controls after a 75-g oral glucose tolerance test (OGTT; see Ref. 14). Analysis of the concentration data of the three peptides with a mathematical model clearly demonstrated that the time course of endogenous amylin release could be predicted from the kinetics of insulin and C-peptide. However, the kinetics of amylin appeared to be slower than that of insulin and similar to that of C-peptide (32). The importance of comparing the kinetics of the three peptides under identical conditions is substantiated by the fact that the molar ratio of amylin to insulin is often used as a marker of β -cell release in pathological situations, even in dynamic conditions (1, 20, 22). If the two substances exhibit different kinetics, however, the use of their molar ratio is correct only during steady-state conditions. Amylin is deficient in insulin-dependent diabetes mellitus (11) and late non-insulin-dependent diabetes mellitus (22). Especially in the latter case, the molar ratio of insulin to amylin has often been used as an indicator of relative amylin deficiency. Identification of amylin deficiency might thus be used to identify potential target populations for substitution with amylin or its agonist pramlintide. The possibility of different clearance parameters further warrants a more robust study. In fact, amylin could be able to exert a more prolonged and sustained action than insulin in regulating carbohydrate metabolism by acting on glucose production, gastric emptying, and other yet unknown processes (36). In addition, knowledge of the volume of distribution is a prerequisite to speculate about common sites of action of amylin and insulin.

The purpose of the present study was therefore to elucidate amylin pharmacokinetics, by injecting synthetic human amylin (amlintide) in humans to analyze its disappearance curve, as well as its distribution volume, half-life, and plasma clearance rate.

METHODS

Subjects and Study Design

The study was performed in 9 male, healthy subjects [mean age = 26.0 ± 1.6 yr, body mass index (BMI) = 22.6 ± 0.4 kg/m²] without family history of diabetes. The protocol was reviewed and approved by the Ethics Committee of the University of Vienna. The purpose, nature, and potential risks of the study were explained in detail to the participants before obtaining their written consent. After an overnight fast, subjects reported to the clinic in the morning of the investigation day. One cannula was inserted into an antecubital vein of the nondominant arm for infusion of somatostatin (SRIF; Curamed Pharma, Karlsruhe, Germany) and amylin. A dorsal hand vein was cannulated to facilitate venous sampling of amylin concentrations.

SRIF was administered to suppress endogenous amylin release at a bolus of 1.8 µg/kg at -30 min followed by constant infusion of 0.06 µg·kg⁻¹·min⁻¹ throughout the study, as described previously (24). At time 0 min, a rapid bolus of 50 µg of amlintide (synthetic human amylin; provided by Amylin Pharmaceuticals, San Diego, CA) was injected, and blood samples were collected from the dorsal hand vein at 3, 5, 7, 10, 12.5, 15, 20, 25, 30, 40, 50, 60, 80, 120, and 180 min.

Assessment of amylin levels. Precisely 5 ml of blood were put into vacutainer tubes containing sodium-EDTA and a lyophilized protease inhibitor. The samples were immediately placed on ice, and the plasma was separated by centrifugation at 4°C, 2,000 rpm for 10 min within 20 min after collection. Plasma amylin levels were measured using a monoclonal antibody-based sandwich assay (28) that was subsequently converted into a kit format. The changes include the generation of both lyophilized standards and precoated assay plates to increase kit (Amylin Pharmaceuticals) stability. All plasma samples were diluted 1:1 with sample diluent to minimize recovery differences between individual plasma samples.

In validation studies, the assay had a minimum detectable concentration (mean ± 2 SD of the zero standard) of <2.0 pmol/l. Intra-assay and interassay coefficients of variation were <15%. The accuracy of the assay as judged by amlintide (synthetic amylin) spiked into human plasma is $87.3 \pm 10.4\%$ (mean ± 1 SD) of the expected value. Dilution of plasma samples with the zero standard provided results that were $100.5 \pm 9.2\%$ (mean ± 1 SD) of the expected values.

The antibody (F024) does not cross-react with the glycosylated peptides (amylin-like peptides) or with known homologous peptides (CGRP, calcitonin). It does react with rat and human amylin as well as pramlintide. Because the assay uses two specific antibodies, there is added specificity because the epitope of the detection antibody (F025-27) is at the far COOH-terminal end and requires the amidation for binding.

Data Analysis

Noncompartmental analysis. Individual amylin data were analyzed with the standard noncompartmental approach. The disappearance curve after bolus injection was described by a sum of exponentials

$$c(t) = \sum_{i=1, \dots, p} A_i \exp(-\alpha_i t) \quad (1)$$

where $c(t)$ is the plasma concentration (pmol/l) at time t . Parameters A_i and α_i are characteristic of every single exponential and represent the zero intercept and the elimination rate constant, respectively. The selection of the number of exponentials (p) used in Eq. 1 for fitting the measured plasma disappearance curve of amylin is crucial for the precision of the estimation of noncompartmental parameters. The measured plasma disappearance curve of every single subject was fitted using two and three exponentials, with the monoexponential description excluded because it did not allow a comprehensive description of amylin kinetics. Data fitting was performed by minimizing the sum of squared residuals after log transformation of the data

$$\min \sum_{j=1, \dots, n} \left\{ \log [c(t_j)] - \log \left[\sum_{i=1, \dots, p} A_i \exp(-\alpha_i t_j) \right] \right\}^2 \quad (2)$$

where $c(t_j)$, $j = 1, \dots, N$ represents the amylin concentration measured at N discrete time points t_j . The logarithmic transformation of the data was motivated by the high precision of the amylin assay at low concentrations (2–100 pmol/l; see Ref. 28). To determine whether two or three is the most appropriate number of exponential terms to describe amylin disappearance after bolus injection, the F -test criterion was used (31). Results were also empirically evaluated by their physical plausibility, i.e., the solutions were not considered acceptable if the parameters exhibited coefficients of variations that were too elevated (e.g., coefficient of variation >100%) and/or their estimated values were not physiologically plausible.

Noncompartmental parameters were calculated according to standard pharmacokinetic equations (10). In particular, the half-life ($t_{1/2}$, min), of each exponential component was calculated by

$$t_{1/2} = \ln(2)/\alpha_i \quad (3)$$

the initial distribution volume (V_1 , ml) was calculated as

$$V_1 = Q_0 / \sum_{i=1, \dots, p} A_i \quad (4)$$

where Q_0 is the known administered dose. The conversion factor from grams to moles accounts for the fact that the molecular mass is 3,905 daltons. The plasma clearance rate (CL, ml/min) was calculated as

$$CL = Q_0 / AUC \quad (5)$$

where AUC is the total area under the concentration curve extrapolated to infinity given by

$$AUC = \sum_{i=1, \dots, p} A_i / \alpha_i \quad (6)$$

The total mean residence time of amylin in the system (MRT, min) was calculated as

$$MRT = \left(\sum_{i=1, \dots, p} A_i / \alpha_i^2 \right) / AUC \quad (7)$$

The total distribution volume (V_{TOT} , ml) was then determined by

$$V_{TOT} = CL \cdot MRT \quad (8)$$

The fractional clearance rate of amylin (k , min⁻¹) was defined as

$$k = CL / V_{TOT} = 1 / MRT \quad (9)$$

and will be used for comparing amylin clearance with that of C-peptide and insulin.

Compartmental analysis. An equivalent representation of multiexponential kinetics of amylin is the compartmental structure that assumes distribution of amylin in different pools. Under the hypothesis that elimination occurs from the accessible compartment, i.e., where amylin is injected and measured, the irreversible loss from the compartmental system is equivalent to the clearance determined with the noncompartmental analysis. A support to this hypothesis comes from a recent study that concluded that amylin disappearance seems to occur almost entirely in the kidneys (21, 33). Moreover, the number of compartments is equal to the number of exponentials used to fit the data. Among different possible compartmental structures, we adopted the catenary model shown in Fig. 1 for a three-exponential description of disappearance data. Adaptation to a two-exponential description is straightforward. The compartmental model of Fig. 1 is parameterized in terms of the volumes of the compartments and by the intercompartmental flows, which determine the mass exchange between contiguous compartments as a function of the concentration gradient. The parameters of the compartmental model are related to the parameters of the exponential model at different degrees of complexity. The simplest relationships are the equivalences between the clearance rate and distribution volume of the accessible compartment with parameters CL and V_1 of Eqs. 5 and 4, respectively. The total "compartmental" volume given by the sum of the volumes of the single compartments is equivalent to V_{TOT} of Eq. 8. The volumes of the nonaccessible compartments and the intercompartment flows can be derived from the parameters A_i and α_i of the exponential model (see APPENDIX).

To determine an appropriate normalization of the kinetic parameters with respect to anthropometric characteristics, three commonly adopted criteria were considered [body weight (BW), BMI, and body surface area (BSA)]. In particular, pairwise correlations of individual parameters were calculated with respect to BW, BMI, and BSA.

RESULTS

Noncompartmental Analysis

The average disappearance curve of amylin in the nine subjects is shown in Fig. 2. Regarding the choice of the number of exponentials to describe in each individual amylin disappearance curve, the *F*-test criterion provided probability levels of $P < 0.05$ in eight out of nine subjects and a value of $P = 0.45$ in one subject.

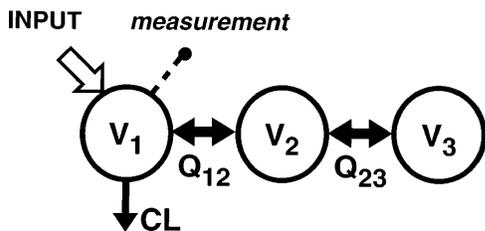


Fig. 1. Compartmental model of amylin kinetics. Amylin injection and measurements occur in the accessible compartment representing plasma. Compartment volumes are initial volume of distribution (V_1), volume of compartment 2 (V_2), and volume of compartment 3 (V_3). Q_{12} and Q_{23} are intercompartmental exchange fluxes from compartments 1 and 2 and from compartments 2 and 3, respectively. CL is amylin clearance rate assumed to occur only from the accessible compartment.

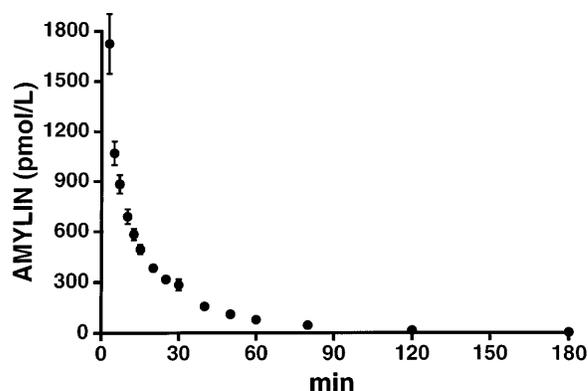


Fig. 2. Average serum amylin concentration after a bolus injection of 50 µg of synthetic human amylin (amlintide) in 9 normal subjects.

These probabilities represent the risk that the increase of the sum of squared residuals for two vs. three exponentials is due to chance alone. Therefore, the use of three exponentials is suitable for all of the subjects and is the best representation of amylin disappearance. The kinetic parameters calculated in each subject from the estimated model parameters A_i and α_i of the three exponentials are reported in Table 1. The average distribution volume and clearance rate normalized to BW were 173 ± 16 ml/kg and 6.2 ± 0.2 ml · min⁻¹ · kg⁻¹, respectively.

Compartmental Analysis

The compartmental model parameters obtained in each subject are reported in Table 2 (only those not already in Table 1 are shown). The analysis for determining the normalization of the kinetic parameters indicated that, when a significant correlation occurred, the correlation with BW was stronger than that with BSA and BMI (data not shown). Thus we evaluated first the correlation between the compartmental parameters and then the correlation between every single parameter and BW (Table 3). It can be noted that a significant correlation was found between each of four

Table 1. Kinetic parameters evaluated with noncompartmental analysis (3 exponentials) of amylin disappearance after an intravenous bolus

Subject	$t_{1/2}$, min			V_1 , liter	V_{TOT} , liter	MRT, min	CL, ml/min	k, min ⁻¹
	1	2	3					
1	2.2	12.3	48.3	5.8	16.0	32.6	492	0.031
2	1.6	15.4	76.1	3.0	16.1	38.3	421	0.026
3	2.5	14.8	69.1	2.5	8.6	23.5	366	0.043
4	2.7	9.0	32.9	8.3	16.8	27.9	603	0.036
5	1.8	10.7	31.6	3.3	8.5	21.8	392	0.046
6	2.6	17.0	62.7	5.2	16.5	33.9	487	0.029
7	2.8	12.5	34.4	4.3	9.3	22.6	412	0.044
8	1.3	8.3	38.4	3.9	12.7	28.2	452	0.036
9	1.2	9.8	28.4	3.1	9.6	20.3	473	0.049
Mean	2.1	12.2	46.9	4.4	12.7	27.7	455	0.038
SE	0.2	1.0	6.0	0.6	1.2	2.1	23	0.003

$t_{1/2}$, half-life for compartment 1 (1), compartment 2 (2), and compartment 3 (3); V_1 , initial distribution volume; V_{TOT} , total distribution volume; MRT, total mean residence time; CL, plasma clearance rate; k, fractional clearance rate.

Table 2. Parameters evaluated with compartmental analysis (model of Fig. 1) of amylin kinetics after an intravenous bolus

Subject	V ₂ , liter	V ₃ , liter	Q ₁₂ , ml/min	Q ₂₃ , ml/min
1	5.6	4.7	720	106
2	7.0	6.2	674	80
3	3.6	2.5	243	33
4	4.7	3.8	610	171
5	3.6	1.6	517	51
6	7.1	4.2	615	63
7	3.6	1.4	367	40
8	4.2	4.6	913	147
9	4.6	1.9	842	66
Mean	4.9	3.4	611	84
SE	0.5	0.5	71	16

Parameters CL and V₁ are the same as in the noncompartmental analysis in Table 1. V₂, volume of compartment 2; V₃, volume of compartment 3; Q₁₂, intercompartmental flow (compartments 1 and 2); Q₂₃, intercompartmental flow (compartments 2 and 3).

parameters, namely the BW, the CL, the V₁, and the intercompartmental flow in compartments 2 and 3 (Q₂₃). In addition, a significant correlation was also found between the volume of compartment 2 (V₂) and the volume of compartment 3 (V₃). Normalization of all compartmental parameters with respect to individual BW markedly changed the above situation. In particular, no correlation was found anymore between any normalized parameters, except between V₂/BW and V₃/BW (correlation coefficient = 0.78, *P* = 0.013). Normalized parameters were V₁/BW = 58 ± 6 ml/kg, V₂/BW = 67 ± 7 ml/kg, V₃/BW = 47 ± 8 ml/kg, intercompartmental flow rate between compartments 1 and 2 (Q₁₂)/BW = 8.4 ± 1.0 ml · min⁻¹ · kg⁻¹, Q₂₃/BW = 1.1 ± 0.2 ml · min⁻¹ · kg⁻¹.

DISCUSSION

Most investigations on amylin and its effects on carbohydrate metabolism have been performed in rats, both in vitro and in vivo (17, 25), whereas the present study directly assessed amylin kinetics in humans by exogenously injecting synthetic human amylin (amlintide) after prior blocking of its endogenous secretion with SRIF. In fact, the potential use of amylin or its

Table 3. Correlations between compartmental parameters of amylin kinetics along with BW

Variable	CL	Q ₁₂	Q ₂₃	V ₁	V ₂	V ₃	BW
CL		0.45	0.78	0.90	0.31	0.31	0.82
Q ₁₂	NS		0.60	0.15	0.36	0.47	0.18
Q ₂₃	0.01 3	NS		0.70	0.13	0.53	0.83
V ₁	0.00 1	NS	0.03 6		0.18	0.23	0.89
V ₂	NS	NS	NS	NS		0.76	-0.14
V ₃	NS	NS	NS	NS	0.01 6		0.13
BW	0.00 7	NS	0.00 6	0.00 1	NS	NS	

BW, body weight. Bold type indicates correlation coefficients. Light-face type indicates corresponding significance levels (NS means *P* > 0.05).

analog pramlintide in the therapy of diabetes requires not only quantitative assessment of the dose/effect but also the investigation of amylin kinetics. In addition, knowledge of amylin distribution, metabolism, and excretion is important in view of an individualized therapy.

The analysis of amylin data after a bolus injection was performed using standard pharmacokinetic techniques to obtain physiological parameters such as the V_{TOT} and the clearance rate. Because a suitable description of plasma disappearance of amylin was obtained by multiexponential curve fitting, as also suggested for kinetics in rats (17), the performance of the two competing models (2 and 3 exponentials) was rated according to statistical criteria to determine the optimal model order. The three-exponential curves provided the best description of the data, which led to the three-pool compartmental representation of amylin kinetics (Fig. 1). This structure had the advantage, over the noncompartmental (multiexponential) analysis, of providing a physiological model that quantified the volumes of three pools of distribution and the exchange fluxes between them. The correlation analysis for determining a suitable normalization criterion showed that compartmental model parameters were more correlated to BW than to other anthropometric characteristics. In particular, it was found that BW allows correlation between most kinetic parameters, which then disappears after normalization, thus suggesting important effects of BW on amylin kinetics. The normalized distribution volumes of the three pools were similar (on average 58, 68, and 47 ml/kg), whereas Q₁₂ was markedly higher than Q₂₃. This supports the validity of the concept that compartments 1 and 2 are related to rapid distribution phenomena between vascular and extravascular spaces, whereas compartment 3 characterizes a remote pool with a slower exchange rate. Another evidence for the possible physiological meaning of the compartments derives from the evaluation of the pool sizes. Assuming that plasma is 7.5% of the total body water (TBW), which in liters is 60% of BW in kilograms (23), plasma volume can be assumed to be roughly 45 ml/kg. This is similar, although slightly lower (*P* = 0.03), to the estimated V₁, which was on average 58 ml/kg. Thus, with good approximation, amylin distributes immediately in plasma and in other fluids with a fast exchange rate. Assuming that plasma and interstitial fluids account for 25% of TBW (23), V_{TOT} is ~150 ml/kg, which was not different (*P* = 0.19) from V_{TOT} of amylin (173 ml/kg). This distribution space compares well to that of insulin (157 ml/kg; see Ref. 30), allowing the conclusion that amylin, as insulin, distributes in the interstitial volume of muscle, adipose tissue, and well-perfused organs in rapid equilibrium with plasma, such as heart, kidneys, gut, and liver. With respect to this correspondence and accepting that V₁ is plasma volume, we conclude that the distribution volumes V₂ and V₃ represent a partitioning of the interstitial fluid volume. The correlation between V₂ and V₃, both with and without normalization to BW, showed that this partitioning follows a linear relationship. The lack of a

statistical correlation between V_2 , V_3 , and BW may be due to the limited number of subjects of our lean study group. Thus the three compartments of the amylin model likely represent plasma (V_1) and the interstitial fluids, partitioned in fast (V_2) and slowly (V_3) exchanging pools.

Because amylin is cosecreted with insulin (12, 13) and therefore with C-peptide, it is interesting to compare the kinetics of the three peptides in a similar situation. The clearance rate of amylin ($6.2 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) is lower than that of insulin ($12.9 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) calculated from a total of 75 normal subjects in 5 different studies after a single injection of insulin (9). The fractional clearance rate of amylin (0.038 min^{-1}) is comparable, although slightly smaller, to that reported for C-peptide ($0.053\text{--}0.072 \text{ min}^{-1}$; see Ref. 29), whereas it is markedly lower than that of insulin ($0.10\text{--}0.20 \text{ min}^{-1}$; see Ref. 5). This indicates that the commonly used insulin-to-amylin molar ratio does not reflect the correct relationship of the two peptides in non-steady-state conditions (32). These results also confirm the clearance rate of endogenously produced amylin during an OGTT obtained by mathematical modeling (14) and can be considered a validation of that model (32). The MRT, which is strictly related to the clearance rate when V_{TOT} does not change, is obtained as the ratio of V_{TOT} over the clearance. Thus the higher MRT of amylin (28 min) compared with that of insulin (14.1 min; see Ref. 30) and C-peptide (16.4 min; see Ref. 29) again supports the lower clearance of amylin. The similar clearance rate of amylin and C-peptide supports our assumption from previous studies that amylin might be cleared predominantly by the kidneys (21, 33). Our data have been collected in lean, healthy subjects with apparent normal kidney function. We have, however, no data on the clearance of amylin in elderly, obese patients, who represent a target population for the therapeutic administration of the amylin agonist pramlintide. Because glomerular filtration rate decreases in elderly people, one could speculate that amylin or pramlintide might exert a prolonged action in these subjects.

In this study, we had to administer SRIF in a dose that is able to suppress pancreatic hormones. SRIF could change renal blood flow and thus influence amylin kinetics. The similar clearance rate for amylin (5.7 compared with $6.2 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in our study) in a paper by Bretherton-Watt and co-workers (2) during high-dose infusion of amylin without SRIF administration, however, suggests that the potential effects of SRIF do not profoundly affect our calculations.

The similar distribution volumes of amylin and insulin found in our study are a prerequisite for the suggested common sites of action as postulated from animal experiments (7, 19, 37). The lower clearance and fractional clearance rates as well as the higher MRT of amylin compared with those of insulin might favor an extended mode of action after oral glucose ingestion.

In conclusion, we found evidence that amylin distributes equally to insulin in plasma and interstitial fluids.

The lower clearance rate of amylin, which is close to that of C-peptide, as well as the higher MRT compared with insulin, indicate that the commonly used insulin-to-amylin ratio is not applicable under non-steady-state conditions.

APPENDIX

The most common mathematical representation of a catenary model, like that of Fig. 1, describing the kinetics of a substance after bolus injection is given by the following system of differential equations

$$\begin{aligned} dx_1/dt &= -(k_{01} + k_{21})x_1 + k_{12}x_2 & x_1(0) &= \text{dose} \\ dx_2/dt &= k_{21}x_1 - (k_{12} + k_{32})x_2 + k_{23}x_3 & x_2(0) &= 0 \\ dx_3/dt &= k_{32}x_2 - k_{23}x_3 & x_3(0) &= 0 \end{aligned} \quad (A1)$$

where x_1 is the mass of the accessible compartment, such that the measured concentration is $c(t) = x_1/V_1$, dose is the amount of substance administered by bolus injection, and parameters k_{ij} represent the fractional exchange rates between the contiguous compartments i and j . The complex relationships that describe the equivalence between the k_{ij} parameters and those from the multiexponential model (Eq. 1) are reported in any pharmacokinetics textbook (10) and have been used to calculate the equations that follow. In this study, we adopted a compartmental representation that uses the intercompartment flows Q_{ij} and the volumes V_i instead of k_{ij} as physiological parameters. The relationships among Q_{ij} , V_i , and parameters k_{ij} of Eq. A1 derive by the definition of fractional clearance, i.e.

$$\begin{aligned} k_{01} &= \text{CL}/V_1 \\ k_{21} &= Q_{12}/V_1 \\ k_{12} &= Q_{12}/V_2 \\ k_{32} &= Q_{23}/V_2 \\ k_{23} &= Q_{23}/V_3 \end{aligned} \quad (A2)$$

which yield the following relationship

$$\begin{aligned} Q_{12} &= V_1 k_{21} \\ Q_{23} &= V_1 k_{32} k_{21} / k_{12} \\ V_2 &= V_1 k_{21} / k_{12} \\ V_3 &= V_1 k_{32} k_{21} / (k_{23} k_{12}) \end{aligned} \quad (A3)$$

for intercompartment flows and compartment volumes of the model of Fig. 1.

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