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Arginine Pharmacokinetics in Humans Assessed with an Enzymatic Assay Adapted to a Centrifugal Analyzer

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Arginine is used in supra-physiological concentrations as an insulin secretagogue, in both in vitro and in vivo studies. To investigate the pharmacokinetics of arginine in humans, we have developed a rapid, automated assay of arginine in serum, based on our manual enzymatic method (Clin Chim Acta 1988,176;185–94). The limit of linearity of the automated assay was an arginine concentration of 3 mmol/L. Within-run CVs for Ortho control sera with added arginine were 5.5%, 0.8%, and 0.7% at concentrations of 0.16, 1.30, and 2.50 mmol/L, respectively. After 30 min of primed continuous infusions with arginine at infusion rates of 3, 9, 15, and 21 mg/kg per minute, mean (± SEM) arginine concentrations in serum from eight volunteers were 1.17 ± 0.08, 3.44 ± 0.21, 6.84 ± 0.58, and 9.25 ± 0.39 mmol/L, respectively, well within the range of arginine concentrations shown (in vitro) to stimulate insulin secretion. Metabolic clearance of arginine was ~11 mL/kg body wt per minute. For the lowest three infusion rates, the half-life ($t_{1/2}$) of arginine was ~15 min and the volume of distribution (V_d) was ~290 mL/kg. At the highest infusion rate, $t_{1/2}$ was significantly increased (27.3 ± 3.1 min), owing to an increased V_d (446 ± 83 mL/kg).

Arginine has been extensively used as a potent insulin secretagogue in both in vitro and in vivo experiments (*1–4*). Its use leads to a dose-dependent, biphasic release of insulin in the presence of glucose (*1*). To our knowledge, determinations of serum arginine concentrations during various infusions of human subjects with arginine have not been published, and data about the pharmacokinetics of this amino acid are scarce. Therefore, one cannot make accurate comparisons between the effects of arginine in vitro and in vivo.

Recently, a specific and accurate kinetic method for determining arginine concentrations in serum was developed (*5*), based on the reaction of arginine with ATP, catalyzed by the enzyme arginine kinase (EC 2.7.3.3). Because this method is suited for the analysis of multiple serum samples, we have adapted it to a centrifugal analyzer.

This study was designed to (*a*) assess the performance of the automated kinetic procedure, (*b*) determine the concentrations of arginine in serum of healthy volunteers achieved during various primed continuous infusions with arginine, and (*c*) calculate the pharmacokinetics of arginine.

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Materials and Methods

Subjects. Eight healthy young men took part in this study, ages 24 (SD 1.1) years, whose mean percentage of ideal body weight was 100 (SD 8)%. Throughout the duration of each study the patient lay quietly on a bed.

The study (part of a larger study of insulin secretion in humans) was approved by the Ethical Committee of the Free University Hospital, and all individuals gave informed consent.

Protocol. Each subject was studied on four separate occasions after an overnight fast. Into each arm an intravenous infusion line was inserted, in an antecubital vein. One line, kept patent by means of a slow drip of isotonic saline (NaCl, 150 mmol/L) was used exclusively for blood sampling; the other line was used for infusion.

Arginine hydrochloride was administered as primed continuous infusions at a rate of 3, 9, 15, and 21 mg per kilogram of body weight per minute during 30 min, in no set order. The dose generally assumed to stimulate insulin secretion maximally is 15 mg/kg per minute (*6*). The infusions were started by a bolus, given over the first 2 min, of one-eighth of the total dose. For an average 70-kg subject, this is equivalent to boluses of 0.8 (3.8), 2.4 (11.4), 4.0 (19.0), and 5.6 g (26.6 mmol), respectively. Blood was sampled at time zero to determine basal concentrations of arginine. Additional samples were obtained 5, 20, 30, and 45 min after the infusion of arginine had been started.

Chemicals. Arginine hydrochloride for infusion was purchased from Ajinomoto Co., Tokyo, Japan. Arginine kinase, derived from lobster-tail muscle, was from Sigma Chemical Co., St. Louis, MO. Other chemicals were all reagent grade.

Reagents and standards. The working reagent contained 7.7 mmol of ATP disodium salt, 3.5 mmol of magnesium chloride, 0.88 mmol of NADH disodium salt, 5.3 mmol of phospho*enol*pyruvate, 17 kU of lactate dehydrogenase (EC 1.1.1.27), and 8.4 kU of pyruvate kinase (EC 2.7.1.40) per liter. The working reagent and the arginine kinase solution were prepared in 0.19 and 0.10 mol/L solutions of glycine buffer (pH 8.6 at 30 °C), respectively, as described (*5*). Working standards were prepared by diluting a stock standard of arginine hydrochloride, 4 mmol/L, in distilled water.

Analytical methods. Blood was sampled in tubes without anticoagulant and allowed to clot for 20 min at room temperature. Serum was separated by centrifugation and stored at −20 °C until assayed.

The protocol for use with the Multistat III centrifugal analyzer (Instrumentation Laboratory, Lexington, MA) was as follows. The sample syringe of the Multistat Loader delivered 10 μL of calibrator, control, or patient's sample and 90 μL of the working reagent into the sample holes of the rotor. The reagent syringe added 10 μL of the arginine kinase solution and 100 μL of distilled water to each

reagent porthole. The "second reagent" and "reagent/diluent" switches were set on "on" and "diluent," respectively. Analyzer settings were: filter 340 nm, start mode 1, pre-incubation time 240 s, delay interval 10 s, and data interval 170 s. Two data points were collected and the change in absorbance was monitored at 30 °C.

Quality control. Precision studies were performed with sera obtained during the infusion experiments and with human control sera (Ortho Diagnostic Systems, Raritan, NJ) supplemented with arginine to give above-physiological values for arginine. We tested the accuracy of the Multistat method by a method-comparison study, using our manual assay for serum arginine (*5*) and an automated amino acid analyzer (Chromaspec; Rank Hilger, U.K.) technique for deproteinized samples.

Calculations of pharmacokinetics of arginine. The metabolic clearance rate (MCR), expressed as mL/min per kilogram body weight, was calculated as the steady-state concentration of arginine in serum at the end of the infusion divided by the infusion rate. The half-life ($t_{1/2}$) was deduced by log transformation from the concentration of arginine in the two blood samples drawn after the infusion was stopped. The apparent distribution volume (V_d) for arginine was calculated according to the following formula (*7*): V_d (mL/kg) = MCR × $t_{1/2}$/0.693.

Statistics. Values are expressed as the mean ± SEM, unless otherwise indicated. Student's *t*-test for paired samples was used to show statistical significance. Differences with $P < 0.05$ were considered significant. Deming's debiased regression (*8*) was used for the method-comparison study.

Results

Arginine assay characteristics. The assay was performed according to the manual (two-point) kinetic protocol previously described (*5*), with minor modifications. The sample-to-working reagent ratio was 1:10 (manual assay 1:11). The Multistat could make the first reading of the absorbance (*A*) 10 s after mixing the reaction components (manual assay: 60 s). In both methods, however, the second reading was taken 180 s after mixing. The assay response varied linearly with arginine concentration up to 3 mmol/L, as assessed by use of serial dilutions of control sera. For an arginine concentration of 0.5 mmol/L, the difference in absorbance between the first and second reading was 0.080. Table 1 summarizes the results of the imprecision study. We checked the accuracy of the method by analyzing serum samples with the Multistat and with two comparison methods. Acceptable agreement was obtained between results by the present assay (y) and the manual method ($y = 0.95x + 0.02$, n = 18, $r = 0.989$) and the amino acid analyzer technique ($y = 0.96x + 0.01$, n = 21, $r = 0.975$).

Analysis for arginine in serum from normal volunteers. Figure 1 illustrates the concentration–time profiles for serum arginine in the eight volunteers. The mean ± SEM arginine concentrations measured after the bolus administration were 0.76 ± 0.05, 1.99 ± 0.13, 3.26 ± 0.46, and 4.53 ± 0.26 mmol/L, for the four doses, respectively—significantly exceeding the baseline values (0.11 ± 0.01 mmol/L, $P < 0.01$). At the end of the continuous infusions, arginine values were 1.17 ± 0.08, 3.44 ± 0.21, 6.84 ± 0.58, and 9.25 ± 0.39 mmol/L, respectively, and declined afterwards.

Arginine pharmacokinetics. The MCR of arginine was ~11 mL/min per kilogram, decreasing slightly at the highest infusion rates (Table 2). This decrease was statistically significant only for the infusion rate of 15 mg/kg per minute. The $t_{1/2}$ for serum arginine was ~15 min, the V_d ~290 mL/kg, as assessed from the three lowest infusion rates. At the highest infusion rate $t_{1/2}$ and V_d were respectively increased to 27.3 ± 3.1 min and 446 ± 83 mL/kg ($P < 0.01$).

Table 1. Precision of the Multistat Method for Arginine in Serum

Arginine, mmol/L		
Mean	**SD**	**CV, %**
Within-run[a] (n = 16 each)		
0.16	0.01	5.5
1.30	0.01	0.8
2.50	0.02	0.7
Between-run[b] (n = 8 each)		
0.75	0.02	2.3
1.78	0.04	2.0
Day-to-day[b] (n = 45) each)		
0.78	0.06	7.9
1.80	0.10	5.5

[a] Human serum with added arginine. [b] Ortho controls.

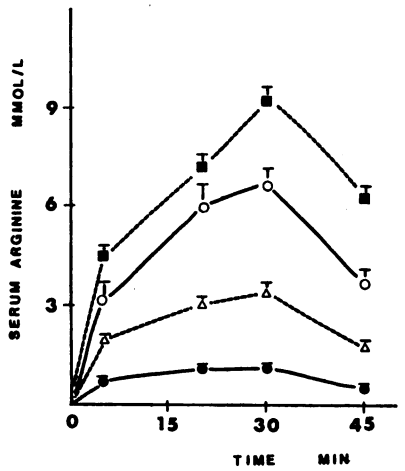

Fig. 1. Time–concentration curves for serum arginine (mean ± SEM) during infusions with arginine at rates of 3 (●), 9 (△), 15 (○), and 21 (■) mg/kg per minute in eight healthy volunteers

Discussion

These data demonstrate that our enzymatic assay for serum arginine can be performed easily and accurately in a centrifugal analyzer. Multiple samples are analyzed at the

Table 2. Metabolic Clearance Rate, Half-Life, and Distribution Volume (Mean ± SEM) of Arginine in Serum from Eight Healthy Volunteers at Four Different Infusion Rates of Arginine

	Arginine infusion, mg/kg per minute			
	3	**9**	**15**	**21**
MCR, mL/min per kg	12.3 ± 0.8	12.8 ± 0.8	10.6 ± 0.7[a]	10.8 ± 0.7
$t_{1/2}$, min	14.6 ± 1.0	15.9 ± 0.9	17.8 ± 1.9	27.3 ± 3.1[a]
V_d, mL/kg	261 ± 26	327 ± 37	274 ± 38	466 ± 83[a]

[a] $P < 0.05$ vs values at lowest infusion rate.

same time in the same run with lower CVs than those obtained with the manual procedure (5). If necessary, heparinized plasma may be substituted for serum. EDTA-treated plasma gave an average increase in arginine concentrations of ~15%.

Using the present method, we could assay serum samples having a wide range of arginine concentrations, obtained after infusions with arginine. The arginine concentrations are well within the range of concentrations used in in vitro experiments (1). If arginine (or any other compound) is to be administered to volunteers or patients safely, one needs knowledge about the therapeutic and side-effects of the compound, and about its pharmacokinetics, to calculate what doses to administer.

We found that the MCR of arginine in humans is ~11 mL/kg per minute and is almost constant over the range of infusion rates tested, with a slight decrease at the highest concentrations.

We also found that the $t_{1/2}$ of arginine in humans is ~15 min for the three lowest infusion rates; this $t_{1/2}$ value was increased to 27 min at the highest infusion rate used. Roberts and Smith (9), determining serum arginine concentrations in five healthy men during infusions with arginine at 15 mg/kg per minute, found a broad range of $t_{1/2}$ values between 30 and 80 min (as deduced from their Figure)—considerably longer than we found in our studies. However, the arginine assay they used had greater imprecision: the within-run CVs at arginine concentrations of 1.1 and 2.2 mmol/L were 15.9% and 9.7%, respectively, compared with <1% in our assay. We did find a longer $t_{1/2}$ for the highest infusion rate, attributable to the larger V_d (446 ± 83 mL/kg).

The V_d calculated for the infusion rates, including the rate that is generally assumed to stimulate pancreatic secretion maximally (15 mg/kg per minute), is about 290 mL/kg, only slightly higher than that reported (10) for the extracellular volume (200 mL/kg). We conclude that arginine is therefore mainly distributed over the extracellular space. Because the V_d was increased at the highest infusion rate, a larger proportion of the arginine present apparently is taken up by the tissues at this infusion rate. Presumably, this is a truly reversible process, as shown by the unchanged MCR and the increase in the $t_{1/2}$.

In conclusion, our data demonstrate the reliability of the automated version of our enzymatic assay of arginine in serum in terms of imprecision, accuracy, and speed. Moreover, we have found that the pharmacokinetics of arginine in humans are practically constant over the range of infusion rates generally used. The V_d for arginine is increased only at a very high infusion rate.

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Dissociation of Immunocomplexes by Ionic Shock for the Development of Immunosensors: Application to Measurement of α_1-Fetoprotein

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We report here our experimental results on the reaction rate of immunological complexes and on potential repeated use of membranes as a result of dissociation of the complexes between α_1-fetoprotein and catalase-labeled antibodies by different buffers with various ionic strengths. The measurement consists of an immunological process and an enzymatic reaction. The protein membrane activated by glutaraldehyde for the immobilization of antibodies is fixed over an oxygen electrode. After incubation with antibody/α_1-fetoprotein and antibody/α_1-fetoprotein antibodies coupled to catalase, the reaction medium is introduced into a continuous-flow cell. Oxygen production by the catalase is measured on-line, with the electrode in contact with hydrogen peroxide. This response is correlated to the α_1-fetoprotein concentration of the sample. We show a typical calibration curve between 0.5 and 120 μg/L. Replicate (n = 20) equilibrium measurement with the same membrane gave a CV of 2.2%. The reversible immunochemical sensor has been tested for 30 measurements without significant loss of activity.

Numerous different designs have been proposed for immunoassays during the last 10 y. Immunological biosensors perhaps owe their origin to early attempts conducted with

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