Metabolism of Creatinine in Vivo

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Two forms of radiolabeled creatinine were used in this study, [carbonyl-14C]- and [amidino-14C] creatinine, the latter being synthesized in this laboratory. These labeled compounds were given intravenously to rabbits. Specimens of plasma, urine, feces, and selected tissues were collected. These were liquid-chromatographed and the separated components compared for radioactivity and identity. This first use of amidino-labeled creatinine revealed the metabolic conversion of small amounts of creatinine to an unidentified non-basic compound and guanidinobutyrate in normal rabbits and to guanidinopropionic acid and arginine in a rabbit that had a low creatinine clearance. A mechanism is suggested for this metabolic process. The proposed pathway may be a major route of elimination of creatinine in renal failure.

Additional Keyphrases: guanidinobutyrate · kidney · disease · chromatographic resolution of guanidino compounds

One would expect that creatinine production would continue at the normal rate in the uremic patient (1), because muscular deterioration is not a feature of this disorder. However, in the anephric subject it reaches a steady-state concentration in plasma, which indicates that there are mechanisms for removal of creatinine other than by renal excretion. Feedback inhibition of creatinine production has been suggested (2), but without direct evidence. It has been proposed (3) that creatinine may be converted back to creatine and to methylguanidine, but this hypothesis has not been verified. Moreover, on the basis of in vitro demonstration of anaerobic degradation of creatinine by colonic contents of normal and uremic subjects, an enteric cycling hypothesis has been suggested (4, 5). Although the mechanism of urea transfer in the gut has been well studied, corresponding data for creatinine are scant.

Most studies of creatinine disposition have used creatine or creatinine radiolabeled at the carbon in the methyl or carbonyl group. Because the methyl group is likely to be lost or exchanged, it is the least useful to use in studying the in vivo metabolism of this molecule. The carbonyl label is of value only if the amidino group remains attached to the acid chain. If the amidino group is transferred, the label would be left on the sarcosine residue, which may be metabolized via several pathways.

In light of previous studies that have suggested that several guanidino derivatives may be accumulated in uremic patients (6-9) it was of special interest to trace the amidino group of creatinine. This initial attempt to do so was directed toward comparing the distribution and metabolism of two radio-tagged creatinine forms, one with the label in the amidino group and the second with the label in the carbonyl group.

Materials and Methods

Significant proportions of [carbonyl-14C] creatinine, obtained commercially, were found to be creatine. [Carbonyl-14C] creatine, properly labeled, was also obtained commercially (ICN Pharmaceutical Inc., Irvine, CA 92715). Liquid chromatography showed this to contain 81.5% of the radioactivity in the form of creatine, 0.4% as creatinine, and 13.2% as glycochymine. An unidentified substance, which was not retained in the column, represented 4.9% of the radioactivity.

[Amidino-14C] creatine was prepared according to an adaptation of the procedure of Bloch and Schoenheimer (10). Briefly, sarcosine was dissolved in water containing one drop of concentrated ammonium hydroxide. To this solution was added unlabeled and 14C cyanamide in 20 mL of diethyl ether and the mixture was kept at room temperature for 48 h. Labeled creatine hydrate in crystalline form was collected and washed with ether. Liquid chromatography showed 78% of the radioactivity to be in the form of creatine and 2.6% as creatinine. A further 19.4% was not retained on the column but was removed by repeated washing with diethyl ether; hence, we assumed it to be dimerized or unreacted cyanamide, or both.

The labeled creatine was then converted to creatinine as follows. A mixture of 0.9 mg of labeled creatine hydrate with 0.55 mL of concentrated hydrochloric acid and 0.15 mL of water was placed in an autoclave for about 4 h at 120 °C and 2.02 MPa (20 atm) pressure. The solution was then chilled at 0 °C in an ice bath, and neutralized with 2 mol/L sodium hydroxide. Crystals of creatinine precipitated and were filtered off, recrystallized, and dried.

A chief difficulty of any investigation related to creatinine, one that has not been surmounted, is the ease with which creatine and creatinine undergo interconversion. The acidity of the medium is generally agreed to be the chief factor governing the direction and extent of this. An increased acidity, particularly at higher temperatures, accelerates the rate of transformation of creatine to creatinine, but even at low pH (pH 1 and 120 °C) an equilibrium appears to be reached, with a molar ratio of 1/40 for creatine/creatinine. Therefore, one cannot assume a 100% creatinine solution, especially at a pH near physiological. However, in this comparative study both forms of creatine were subjected to the same condition of acid hydrolysis and hence had about the same proportion of creatine. Repeated recrystallization removes most impurities, if not all, and storing the dry crystals in a desiccator under reduced pressure, in the freezer, helps stabilize the 90-95% creatinine formed. Solutions for injection were prepared just before use.

For all chromatographic studies we used a Perkin-Elmer Model 1210 "high-pressure" liquid chromatograph. A stainless-steel column (9 mm × 30 cm) packed with a strong cation-exchange resin (Aminex A-4; Bio-Rad Labs., Richmond, CA 94804) was used. Samples were injected at pH 3.25, and a linear pH gradient was automatically run from pH 3.25 to 11.0, with 0.2 mol/L citric acid in one pump and 0.2 mol/L NaOH in the second. The time for this gradient to be completed was 160 min; the flow rate was 2 mL/min.

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We found that standard mixtures of the biologically occurring guanidino compounds were well resolved and could be collected separately in 4-mL fractions of the column effluent. A similar procedure has been described by Perez et al. (7). An aliquot of each such fraction was measured for radioactivity by liquid scintillation counting. Creatinine was measured in a 1-mL aliquot by use of sodium picrate. We also treated 2-mL portions with Voges-Proskauer reagent (11), to obtain colored products of guanidines. Peak identification was based on retention times as compared with those for authentic standards.

Animals and treatment of biological samples: We used male New Zealand White rabbits, 3–3.5 kg, 15–20 weeks old. Tracer quantities of the creatinine (specific activity, 23 mCi/g) were injected intravenously, and blood was sampled and urine specimens were collected during the next 6 h. The animals were then placed in a metabolism cage overnight, and feces and urine were collected during this interval. The next day the animals were killed and various tissues were sampled.

Plasma proteins were removed by use of ultrafiltration cones (Amicon Corp., Model 26-50-59A). Tissues and feces were homogenized with 10 volumes of water at 0 °C. The homogenates were centrifuged to remove coarser particles and then filtered through ultrafiltration cones as with plasma. A portion of each sample was measured for total radioactivity; the rest was stored at −10 °C until assayed.

Results

Plasma and urine: Chromatographic analysis of plasma and urine samples from the first group of animals (C, C1, C2, C3 = 5.82 ± 0.31 mL/min) that received 17 μCi of [carbonyl-14C]creatinine showed that some radioactive material was eluted soon after the void volume of the column. Its chemical nature is unclear, but it could represent small neutral or acidic molecules; for convenience, it is referred to here as "initial peak(s)" (IP). This material was detected in both plasma and urine. The rest of the radioactivity was in the form of creatinine.

The second group of animals (C3, C4, C5) received 17 μCi of [amidino-14C]creatinine. The chromatographic profiles of typical plasma and urine samples are illustrated in Figures 1 and 2. As shown in the plasma chromatogram, in addition to IP, only one other radioactive peak was present, and it corresponded to creatinine. For urine samples, however, the chromatographic patterns were different from those for the first group. Urine samples of two animals (C3 and C4; C5 = 5.02 ± 0.4 mL/min) showed a substantial amount of radioactivity present in the form of guanidinobutyric acid. In the urine of the third animal, C6 no radiotagged guanidinobutyric acid was found, but substantial amounts of labeled guanidinopropionic acid and arginine were present. Creatinine clearance by this animal was abnormally low, Ccr = 0.91 mL/min, indicating some renal damage, and the excretion of arginine may have reflected this.

Relatively small proportions of creatinine, 0.72–1.71%, were found in the form of its metabolites in the urine of these animals. After single intravenous injections, the specific activity of creatinine declined from an initial high value immediately after injection to a relatively low one at 2 h. The disposition pattern and related mathematical model have been described elsewhere (12, 13). The principal cause of changes in the specific activity of creatinine in plasma is the relatively rapid uptake of radioactive creatinine into tissue. Cumulative measurements of urine radioactivity showed that about half of the dose can be accounted for as creatinine in about 3 h. In long-term urine collection, in a separate experiment, measurable amounts of radioactivity were found in the form of creatinine after two weeks. At such long times after injection the rate of excretion is probably determined by the slow rate of efflux from tissues—especially muscle. The endogenous creatinine concentrations in serum fluctuated little (0.45–0.5 mmol/mL), demonstrating that steady-state conditions prevailed during the experiments.

In Table 1, we compare the amount of creatinine and its metabolites in various tissues of the second group of animals. We could identify only creatine and arginine. Analysis of the same tissues from [carbonyl-14C]creatinine-treated rabbits showed labeled creatinine to be the only identifiable radioactive compound present. In liver from animals treated with amidino-labeled creatinine we observed two unidentified radioactive substances, a nonbasic molecule(s) and a substance that was eluted between pH 4 and 5 and did not react with the guanidine reagent.

Muscle and other tissues: The radioactivity was more concentrated in muscle than in other tissues (Table 1). As shown, creatine and arginine were the only two radioactive metabolites present in muscle from the second group of animals. In muscle from the first group, creatinine was the only radiolabeled compound present.

Feces: The 24-h feces, extracted and chromatographed as described in Materials and Methods, showed unchanged creatinine as the sole radioactive amidino compound. An
unidentified metabolite(s) without an amidino group was present in substantial quantities. A total of 0.216% of radioactivity injected was excreted by way of the feces in 24 h.

**Discussion**

From our results we conclude that the rabbit metabolizes a small proportion of creatinine. The basic metabolites were chromatographically identical with guanidinobutyric acid in normal animals, and with guanidinopropionic acid and arginine in the rabbit that had renal impairment. These metabolites were not detected when [carbonyl-14C]creatinine was used. This explains why previous investigators have not detected this metabolism: the radioactive atom must be in the amidino group of the ring if the product of conversion is to be detected.

It was surprising that the chromatographic pattern for muscle from [amidino-14C]creatinine-treated animals did not more closely resemble that observed for [carbonyl-14C]creatinine-treated rabbits. Feces of both groups contained significant amounts of radioactivity in the IP, a non-basic metabolite, and a high proportion of unchanged creatine. Our observation of two unknown peaks in chromatograms of liver contrasts with previous in vivo studies in which only creatinine was seen. Here also, the apparent discrepancy seems to be related to our use of [amidino-14C] rather than [carbonyl-14C] creatinine.

One could interpret these data to mean that creatinine enters the urea cycle via formation of carbamoyl phosphate. Evidence consistent with this view has previously been obtained and this suggestion made by several authors (14, 15) in studies that showed the role of phosphate in the breakdown of creatinine and conversion of sarcosine, ammonia, and carbamoyl phosphate. The carbamoyl phosphate then enters the urea cycle to form citrulline, proceeding to arginine. The arginine formed, now bearing the amidino group from creatinine, may split to form urea or may participate in protein synthesis or transamidination reactions. Arginine:H2N-R amidinotransferases may then participate in the biosynthesis of creatine and other guanidino compounds.

It was believed that the proposed pathway would then become a major route of elimination of creatinine molecules in renal failure. Clearly, it would not rule out the possibility of enteric cycling of creatinine (4, 5) and it is consistent with the present understanding of the effects of chronic renal failure, such as increased concentrations in plasma of phosphorus (16), citrulline (17, 18), and glycine (10, 17, 18), and normal concentrations of arginine in plasma (17-22). In addition, it would suggest that creatine present in the muscle is not formed only by simple hydration of creatinine, as one might suspect, but also might arise via metabolism and re-synthesis from arginine and glycine. This is confirmed by absence of radiolabeled creatine in muscle when [carbonyl-14C]creatinine was administered. The IP in chromatograms of plasma, urine, feces, and liver are currently unidentified, but it is tempting to speculate that part of such peak(s) in [amidino-14C]creatinine-treated animals is urea, in [carbonyl-14C]creatinine-treated animals it is sarcosine, and in feces it would be the products of bacterial degradation. We believe the substance(s) in liver samples that was eluted between pH 4 and 5 and did not bear an amidino group represents two or more components of the ornithine-urea cycle.

The concept of in vivo metabolism of creatinine has widespread importance. It may serve as a basis for understanding the origin of various guanidino compounds found in uremia (6, 8, 9); it can be incorporated into the creatinine model (12, 13), which then can be applied to uremic subjects as well as normal; and it may serve to elucidate the mechanism and value of alpha amino acids and alpha keto acids (23) in therapy of uremia.

**References**