Transamidination in the Nephrectomized Rat

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(Received for publication, April 24, 1959)

Creatine is synthesized by the rat from arginine, glycine and methionine by the following enzyme-catalyzed reactions:

\[ \text{L-Arginine + glycine} \rightarrow \text{guanidinoacetate + L-ornithine} \]  

(1)

\[ \text{Guanidinoacetate + S-adenosylmethionine} \rightarrow \text{creatine + S-adenosylhomocysteine + H}^+ \]  

(2)

Reaction 1 is catalyzed by the enzyme arginine-glycine transamidinase (1), and Reaction 2 by guanidinoacetic acid methyltransferase (2). Until recently, the only site of the enzyme transamidinase in mammals was thought to be the mammalian kidney (1, 3). In brief footnotes, Horner et al. (4) mentioned preliminary experiments which indicated that an enzyme which catalyzes the reversal of Reaction 1 is present in rat liver, and Walker (5) mentioned the detection by means of a canavanine-orinthine assay system of weak transamidinase activity in mammalian thymus and testes. The first data which show that a major extrarenal source of the enzyme exists in mammals were presented quite recently by Walker (6). This investigator detected, in vitro, transamidinase activity in the pancreas of the dog. Interestingly enough, the specific activity of the pancreatic enzyme, on a wet weight basis, was approximately 5 times higher than that of dog kidney.

In the present report, evidence is presented that creatine can be synthesized by rats that have been bilaterally nephrectomized, thus the existence of an extrarenal site(s) of transamidination in this animal is indicated. Even more important, the results show that extrarenal transamidinase activity may be of considerable significance in the synthesis of creatine by the rat.

EXPERIMENTAL

Radioactive Compounds—Glycine-1-C\(^14\) was purchased from the New England Nuclear Corporation, Boston, Massachusetts.

Assay of Radioactivity—All samples were burned to CO\(_2\) which was then assayed as BaCO\(_3\) with a gas flow Geiger-Müller tube.

Animals—Adult albino rats (Wistar) were used for all experiments. The animals were fed a commercial (Purina) diet before and during the experiments. The animals were bilaterally nephrectomized under Nembutal anesthesia by the usual dorsal approach. Immediately after recovery from the anesthetic, 1 mg. of labeled glycine was injected subcutaneously. Food and water were given ad libitum. Except for the rats which died in Experiments 1 and 2, the remainder of the animals were killed by a blow on the head 48 hours after the injection of glycine.

Isolation of Creatine from Muscle—Fifty to 70 grams of skeletal muscle were homogenized with 150 ml. of distilled water for 30 minutes, and the temperature was gradually elevated to 90° during this process. The homogenate was cooled and filtered through cheesecloth. An equal volume of ethanol was then added to the filtrate and the solution was chilled for several hours. The precipitated protein was removed and the clear filtrate was concentrated under reduced pressure to a volume of 50 ml. This solution was placed in a cellulose bag and dialyzed against several 1-l. volumes of distilled water until essentially all of the creatine had dialyzed out, as judged by the diacetyl reaction (7). The dialysate was passed over 10 gm. of Amberlite IR-120 (H\(^+\)) (2-cm. diameter column) at the rate of 8 to 10 ml. per minute. The resin was washed with water, and the creatine eluted with 0.5 N NH\(_2\)OH (8). The eluate was evaporated to dryness in a vacuum. The residue was dissolved in a minimal quantity of boiling water and filtered. An equal volume of ethanol was added and the solution was placed in the cold overnight. The creatine was recrystallized from water-ethanol.

Creatine Zinc Chloride—Creatine was converted to creatinine hydrochloride by refluxing with 1.0 N HCl for 3 hours. The zinc chloride salt was prepared according to the method of Benedict (9). It was recrystallized from boiling water by adding 4 volumes of ethanol.

Degradation of Creatine Zinc Chloride—The compound was degraded by the procedure described by du Vigneaud et al. (10) to obtain the methyl carbon as monomethylamine chloroplatinate.

Analysis of Compounds—Creatine zinc chloride was either analyzed for nitrogen and chloride content, or the purity was determined by colorimetric (Jaß6) analysis. Monomethylamine chloroplatinate was analyzed for platinum. All samples were found to be pure by analysis within ±2 per cent of theory.

Radiopurity—Recrystallization of creatine, creatine pircate, or creatine zinc chloride in the presence of glycine-1-C\(^14\) resulted in a reduction of contaminating activity to negligible quantities. The radiopurity of the isolated creatine was confirmed by noting the constancy of the molar specific activity when creatine was converted first to creatine pircate, then to creatinine hydrochloride, and finally to creatinine zinc chloride. To eliminate the possibility of contamination by traces of glycine containing polypeptides, the isolated creatine (in several experiments) was heated in a sealed tube with 6 N HCl at 110° for 24 hours. Creatine was isolated as the pircate from the hydrolysate and converted to the zinc chloride salt. No loss of activity was detected. The possibility that the labeling of the creatine was due solely to the methylation of pre-existing guanidinoacetic acid by C\(^4\)-methyl groups formed from glycine-C\(^14\) was eliminated by degradation of the isolated creatine (as creatinine). Less than 10 per cent of label was found in the methyl carbon.
**RESULTS**

Glycine-1-C\(^{14}\) was injected into the nephrectomized, sham-operated, and control rats. After 48 hours, skeletal muscle creatine was isolated and assayed for C\(^{14}\). The results are given in Table I. In all cases, the isolated creatine was radioactive. A comparison of the specific activities of the creatine from nephrectomized rats with that of the control rats indicates that roughly 15 to 22 per cent of the guanidinoacetic acid synthesized in these animals could arise from extrarenal synthesis.

**Table I**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Weight</th>
<th>Sex</th>
<th>Survival time</th>
<th>Specific activity glycine injected (A)</th>
<th>Specific activity creatine isolated (B)</th>
<th>W/A $\times$ B.W. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>256</td>
<td>M</td>
<td>37(^h)</td>
<td>1.99 (\times 10^{-3})</td>
<td>1.36 (\times 10^{-3})</td>
<td>6.8</td>
</tr>
<tr>
<td>2</td>
<td>287</td>
<td>M</td>
<td>45</td>
<td>1.99 (\times 10^{-3})</td>
<td>1.58 (\times 10^{-3})</td>
<td>9.1</td>
</tr>
<tr>
<td>3</td>
<td>254</td>
<td>M</td>
<td>48</td>
<td>1.99 (\times 10^{-3})</td>
<td>2.16 (\times 10^{-3})</td>
<td>11.0</td>
</tr>
<tr>
<td>4</td>
<td>254</td>
<td>M</td>
<td>48</td>
<td>1.99 (\times 10^{-3})</td>
<td>2.59 (\times 10^{-3})</td>
<td>13.2</td>
</tr>
<tr>
<td>5</td>
<td>255</td>
<td>F</td>
<td>48</td>
<td>3.75 (\times 10^{-3})</td>
<td>4.19 (\times 10^{-3})</td>
<td>11.4</td>
</tr>
<tr>
<td>6</td>
<td>265</td>
<td>F</td>
<td>48</td>
<td>3.75 (\times 10^{-3})</td>
<td>3.10 (\times 10^{-3})</td>
<td>8.8</td>
</tr>
<tr>
<td>7</td>
<td>269</td>
<td>F</td>
<td>48</td>
<td>3.75 (\times 10^{-3})</td>
<td>2.77 (\times 10^{-3})</td>
<td>8.0</td>
</tr>
</tbody>
</table>

**Discussion**

The methylation of guanidinoacetic acid to form creatine occurs principally in liver tissue (11). The formation of guanidinoacetic acid in the rat from arginine and glycine was believed to occur only in renal tissue. The results reported in this paper clearly demonstrate that guanidinoacetic acid can be synthesized in rat tissues other than renal tissue. Furthermore, the results indicate that the contribution of extrarenal transamidination to creatine synthesis can be considerable. The sham-operated animals showed a slightly decreased rate of synthesis which probably can be attributed to the trauma of the surgical procedure. It is interesting to speculate that extrarenal transamidination might well be an important factor in maintaining the body creatine pool in the event of the loss of renal mass as a result of disease or injury.

**Summary**

Glycine-1-C\(^{14}\) was administered to bilaterally nephrectomized adult albino rats. Significant quantities of the isotope were found in the isolated creatine of the skeletal muscle; thus the existence of an extrarenal site(s) of transamidination in the rat is indicated. Furthermore, the contribution of extrarenal transamidination to creatine synthesis was found to be considerable.

*Acknowledgments*—The author gratefully acknowledges the technical assistance of Mr. William J. Dougherty.
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