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Research Article

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The Biosynthesis of Guanidinosuccinic Acid by Perfused Rat Liver

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ABSTRACT The metabolic pathway for the synthesis of guanidinosuccinic acid was studied in the rat. Labeled guanidinosuccinic acid was isolated from the urine of animals given L-[guanidino-^{14}C]arginine intraperitoneally but did not appear in the urine after administration of D,L-[guanidino-^{14}C]canavanine. Radioactive arginine and nonradioactive aspartic acid and arginine were infused in the isolated, perfused rat liver. After 20 min, small amounts of both labeled and unlabeled guanidinosuccinic acid and large amounts of urea were detected in radiochromatograms of the perfusate. These results support the theory that guanidinosuccinic acid is formed in the liver from transamidination of arginine to aspartic acid.

INTRODUCTION

Guanidinosuccinic acid (GSA)[1] is a guanidine derivative that has been implicated in the pathogenesis of several uremic manifestations (1–3). Its serum and urinary levels are increased in patients with chronic renal insufficiency which suggests increased production (4).

The metabolic pathway for the synthesis of GSA has not been elucidated. Cohen (5) suggested that a rise in the plasma level of guanidinoacetic acid resulting from renal insufficiency leads to suppression of glycine amidinotransferase and the transfer of the amidino group of arginine to aspartic acid to form GSA. On the other hand, Takahara et al. (6) were unable to form GSA by transamidination from arginine to aspartic acid and suggested that GSA may arise from hepatic degradation of canavaninosuccinic acid.

The objective of this investigation was to evaluate these proposed pathways for the biosynthesis of GSA in the intact animal and in the isolated, perfused rat liver.

METHODS

In vivo studies. Male Sprague-Dawley rats weighing between 150 and 300 g were housed in metabolic cages and allowed free access to food and water. On the day of study, these animals were given intraperitoneal injections of either 15 μCi of L-[guanidino-^{14}C]arginine (sp act 20 mCi/mmol) or D,L-[guanidino-^{14}C]canavanine (sp act 33 mCi/mmol), and 5 mg of the unlabeled compound and a 24-h urine specimen were collected. GSA was determined by ion-exchange column chromatography using the method of Stein et al. (4) and the Voges-Proskauer reaction (7). After chromatography of the urine, the radioactivity of the different fractions was determined by dissolving 1 ml of the effluent in 10 ml of aquasol universal liquid scintillation counting mixture (New England Nuclear, Boston, Mass.) and counting for 10 min in a liquid scintillation spectrometer. Radioactive compounds were obtained from New England Nuclear and Schwartz/Mann Div., Becton, Dickenson & Co., Orangeburg, N. Y.

Liver perfusion. 200-g male Sprague-Dawley rats were anesthetized with sodium pentobarbital intraperitoneally, 5 mg/100 g of body weight and surgically prepared for liver perfusion using a liver perfusion chamber apparatus equipped with a peristaltic pump manufactured by Apparatus Workshop, Vanderbilt University, Nashville, Tenn. The perfusion media (90 ml) consisted of washed dog erythrocytes (20% hematocrit), 4 g/100 ml of fraction V bovine serum albumin (Pierce Chemical Co., Rockford, Ill.) in Krebs' bicarbonate buffer, pH 7.4. The perfusion rate was 6.5 ml/min. The perfusate was oxygenated with humidified oxygen (95% with 5% CO_2) and the system was maintained at 38°C. The duration of perfusion varied from 1 to 2 h. 5-ml samples of perfusate were drawn from the sampling portal at time zero and at various time intervals after introduction of 20 μCi of L-[guanidino^{14}C]arginine (sp act 20 mCi/mmol), 5 mg of nonradioactive arginine and 5 mg of aspartic acid in the perfusion circuit. Viability of the liver preparations was assessed by the arterial to venous color difference, the constant production of bile, and the metabolic conversion of

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[1] *Abbreviation used in this paper:* GSA, guanidinosuccinic acid.

[2] Manuscript submitted for publication.

TABLE I

24-h Urinary Excretion and Specific Activity of Recovered GSA in Animals Given Labeled Potential Precursors

	Experiment no.	GSA		
		Excretion	Radioactivity	Sp act
		μmol/24 h	*cpm/24 h*	*cpm/μmol/24 h*
L-[guanidino-^{14}C]arginine (15 μCi)	1	0.126	5,540	43,968
	2	0.217	10,022	46,184
	3	0.251	9,437	37,598
D,L-[guanidino-^{14}C]canavanine (15 μCi)	1	0.160	0	0
	2	0.423	0	0
	3	0.206	0	0
D,L-[guanidino-^{14}C]canavanine (25 μCi)	1	0.240	0	0
	2	0.303	0	0

the compounds under study. To assess the effects of the perfusion medium per se 5 μCi of L-[guanidino-^{14}C]arginine was incubated at 38°C in the perfusion medium for 2 h. GSA and other guanidine derivatives were isolated by high-pressure liquid chromatography [2] using a cation-exchange resin (Aminex A-5, Bio-Rad Laboratories, Richmond, Calif.). Elution was accomplished with a stepwise sodium citrate buffer system of increasing pH and the effluent was examined for guanidines with the Voges-Proskauer reaction (7). Labeled arginine was purified by this method.

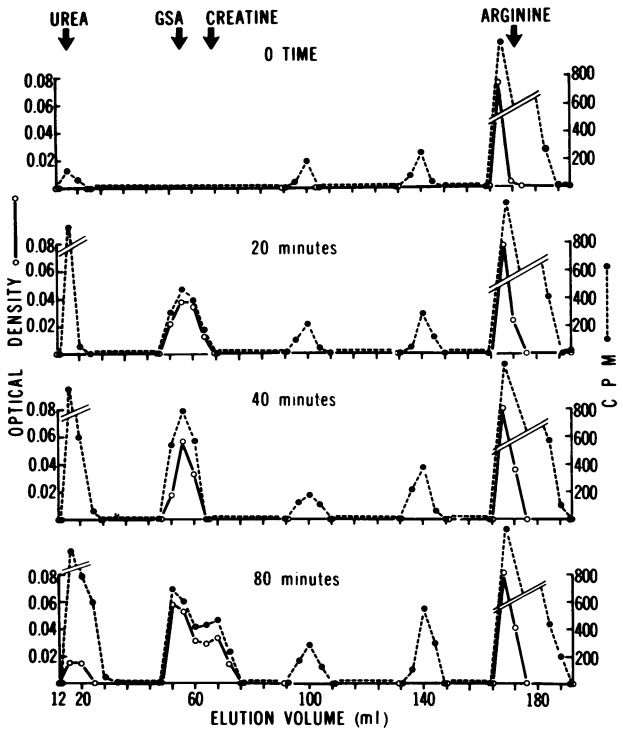

FIGURE 1 Elution profiles on Aminex A-5 of ^{14}C radioactivity (- - -) and optical density of the Voges-Proskauer test (——) at 0, 20, 40, and 80 min after introduction of 20 μCi of L-[guanidino-^{14}C]arginine and nonradioactive arginine and aspartic acid into the liver perfusion system.

RESULTS

In vivo experiments. Labeled GSA was isolated from the urine of all the animals given L-[guanidino-^{14}C]arginine. There was no labeled GSA in the urine of five animals given D,L-[guanidino-^{14}C]canavanine. The 24-h urinary excretion of GSA, radioactivity, and the specific activity of recovered GSA are shown in Table I.

Hepatic perfusion studies. Radiochromatograms of the perfusate at zero time and control incubations at 38°C for 2 h resulted in the formation of three radioactive peaks in addition to that of arginine. One of the peaks corresponded to the elution pattern of urea; the other two were not identified. The results suggest that either spontaneous degradation of arginine occurred or that the perfusate contributed to the degradation of arginine. However, no radioactive peaks were identified between fractions 10 and 20, the region where GSA and creatine are eluted from the column.

Four liver perfusion experiments were performed. A representative radiochromatogram of the perfusate at zero time and at various intervals after the beginning of the experiment is shown in Fig. 1. The cpm of the urea fraction increased from 1.6×10^4 at 20 min to 10×10^4 at the end of the perfusion. By 20 min, in addition to the three peaks previously described, a distinctive radioactive, Voges-Proskauer-positive peak corresponding to GSA had appeared. At 80 min after the beginning of the perfusion, an additional radioactive Voges-Proskauer-positive peak corresponding to the elution pattern of creatine was identified.

DISCUSSION

In the intact animal studies, arginine was found to be a precursor of GSA. On the other hand, there was no urinary recovery of labeled GSA when D,L-[guanidino ^{14}C]-canavanine was given. Studies in the isolated perfused rat liver demonstrated the conversion of arginine to GSA. The presence of small amounts of creatine after 80 min of incubation may be due to a minor but previously unrecognized (8), amount of arginine-glycine transamidinase activity in rat liver.

The present study is in agreement with that of Cohen (5) who suggested that a rise in the plasma level of creatine and guanidinoacetic acid resulting from renal insufficiency leads to suppression of glycine amidinotransferase and the transfer of the amidine group of arginine to aspartic acid to form GSA. He found that GSA is present in the serum of anephric patients and that it disappears from the serum when uremia is complicated with hepatic failure suggesting that the liver and not the kidney is the site of synthesis.

Takahara et al. (6) on the other hand, were unable to detect GSA formation by transamidination from arginine to aspartic acid or asparagine. They found that human liver homogenates can degrade canavaninosuccinic acid to form homoserine and GSA. They speculated that canavaninosuccinic acid may originate in vivo from condensation of canavanine with fumaric acid. In the present study, however, no labeled GSA was found in the urine of animals given labeled canavanine.

These studies demonstrate that arginine is the precursor of GSA, both in the intact animal and in the isolated perfused rat liver. Changes in the hepatic metabolism of arginine in uremia may lead to the increased production of GSA and perhaps to the formation of other toxic guanidine derivatives.

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