Role for Pancreas in Biosynthesis of Creatine.* (23922)

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Two enzymes are involved in the biosynthesis of creatine from its amino acid precursors: arginine-glycine transamidinase and guanidinoacetate methylpherase(1,2). It has been generally accepted that arginine-glycine transamidination occurs only in the kidney; a product of this reaction, guanidinoacetate (glycocyamine), is then methylated in the liver to form creatine. Several mammalian tissues other than kidney have been examined in the past for transamidinase activity, but the results have been uniformly negative (1,3). Recently, in an attempt to clarify the mechanism of transamidinase action, this laboratory undertook a search for other sources of the enzyme. One result of this search was the discovery in *Streptomyces griseus* of a new transamidinase which, unlike kidney transamidinase, does not react with glycine (4). A second result of this search was the discovery of a transamidinase which occurs in high concentration in mammalian pancreas; in this paper certain characteristics of pancreatic transamidinase will be described.

Methods. Dog pancreas and kidneys were removed from the carcass as soon as possible and stored in the frozen state. Ten % tissue homogenates were prepared with a Ten Broeck glass homogenizer in 0.1 M phosphate buffer, pH 7.4, containing 5 mg/ml of ethylenediamine tetraacetic acid (Versene). A more purified enzyme preparation was obtained from pancreas by centrifuging the homogenate and fractionating the supernatant solution with ammonium sulfate at 4°C. The fraction precipitating between 30% and 60% saturation was taken up in buffer plus Versene, dialyzed for 18 hours at 4°C against dilute buffer-Versene, and stored in the frozen state. In the deproteinized incubation mixtures arginine was estimated by the Sakaguchi reaction(5) and hydroxyguanidine was assayed by a pentacyanoammonioferrate method(4). Incubations were carried out in small test tubes in 0.1 M phosphate buffer, pH 7.4. Canavanine sulfate and hydroxylamine hydrochloride were neutralized before use with potassium hydroxide. Synthetic hydroxyguanidine sulfate, used as a standard in the colorimetric assays, was prepared from 1 M S-methylisothiouronium sulfate and 1.5 M hydroxylamine (prepared from hydroxylamine sulfate plus barium hydroxide). The aqueous solution was allowed to react for 24 hours at room temperature in a hood. Hydroxyguanidine sulfate was then precipitated from the reaction mixture by the slow addition of acetone; the filtered precipitate was washed with methanol, followed by acetone. The product was recrystallized from water. The structure of hydroxyguanidine was confirmed by elementary analysis and by reduction to guanidine with palladium and hydrogen. The pK of hydroxyguanidine was found to be approximately 8.1; it is stable in acid solution, but it is less stable in neutral and basic solutions.

Results. Preliminary experiments established that pancreatic transamidinase has the same substrate specificity as kidney transamidinase. Canavanine, arginine, and guanidinoacetate are the most active amidine donors, while ornithine, canaline, and glycine are the most active amidine acceptors(6). It would appear that the synthesis of guanidinoacetate is a major function of pancreatic transamidinase.

Attention was next directed to a comparison of enzyme activities in kidney and pancreas. For this purpose a choice of assay systems from among the various donor-acceptor pairs had to be made. It was recognized that for enzymic rate studies irreversible reactions would be preferable. Previous work in this laboratory had indicated that all transamidinases, whatever their source, have the ability to catalyze (a) an arginine-ornithine exchange reaction(7), and (b) a transfer of the

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TABLE I. A Comparison of Transamidinase Activities of Kidney and Pancreas Homogenates.

Assay system	μmoles product formed/g tissue (wet wt)/hr Kidney	Pancreas
(a) Canavanine, .032 M Ornithine, .022 M Acetone, .4 M 37 mg tissue/ml; 1 hr at 35°C	27 (arginine)	132
(b) Arginine, .033 M Hydroxylamine, .6 M 50 mg tissue/ml; 2 hr at 25°C	2.1 (hydroxyguanidine)	13.3

amidine group of arginine to hydroxylamine, with the formation of hydroxyguanidine and ornithine(8). For assay purposes in this laboratory canavanine, a structural analogue of arginine, is substituted for arginine in Reaction (a). The resulting reversible canavanine-ornithine transamidination can then be made essentially irreversible by trapping one of the reaction products, canaline, with a carbonyl compound such as acetone or pyruvate(9); the other reaction product is arginine, which can be determined colorimetrically. Alternatively transamidinase activity can be assayed by Reaction (b), provided that the system assayed has relatively little arginase activity, since as will be seen, ornithine strongly inhibits Reaction (b).

In Table I the transamidinase activities of dog kidney and dog pancreas are compared, using both assay systems. It is evident that on a wet weight basis transamidinase activity is approximately 5 times higher in pancreas than in kidney. Approximately the same ratio of activities was also obtained for canavanine-glycine transamidination, as estimated from guanidinoacetate formation.

In studies of the mechanism of transamidinase action carried out in this laboratory, attention has recently been focussed upon the role of hydroxylamine as an amidine acceptor. It has been suggested that hydroxylamine might react nonenzymatically with an enzyme-amidine intermediate formed by the interaction of arginine with transamidinase(4). In any event it can be seen from Fig. 1 that high concentrations of hydroxylamine are required to achieve a maximal rate of arginine-hydroxylamine transamidination. (For the experiments of Fig. 1-3, a dialyzed ammonium sulfate enzyme fraction from dog pancreas was used, instead of the more complex homogenate system.) Fig. 2 and Fig. 3 summarize the results obtained from time-course studies of arginine-hydroxylamine and guanidinoacetate-hydroxylamine transamidination reactions, respectively. Both reactions are strongly and specifically inhibited by the amidine acceptors ornithine and glycine. Other closely related compounds which are not amidine acceptors are also not effective inhibitors of hydroxyguanidine formation(4,8).

Discussion. The observation that pancreas is a rich source of arginine-glycine transami-

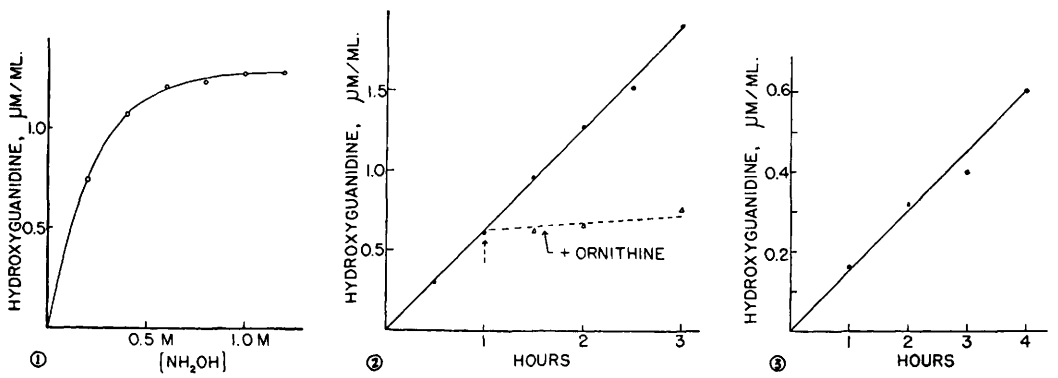

FIG. 1. Rate of arginine-hydroxylamine transamidination as a function of hydroxylamine concentration. Arginine, .033 M; 3 mg enzyme/ml; incubated 90 min. at 21°C.

FIG. 2. Time-course of arginine-hydroxylamine transamidination, showing inhibition by a competing amidine acceptor added to an aliquot at the indicated time. Arginine, .033 M; hydroxylamine, .6 M; ornithine, .03 M; 3 mg enzyme/ml; incubated at 21°C.

FIG. 3. Time-course of guanidinoacetate-hydroxylamine transamidination. Guanidinoacetate, .02 M; hydroxylamine, .6 M; 10 mg enzyme/ml; incubated at 25°C.

dinase suggests that this organ plays an important role in the biosynthesis of body creatine. Whether or not methylation of guanidinoacetate also occurs in pancreas remains to be determined. In any event guanidinoacetate formed in the pancreas and released into the blood stream would have but a short journey *via* the portal vein to the liver, where methylation definitely can take place(2). In contrast to pancreas, the weak transamidinase activities of thymus and testis, briefly noted elsewhere(4), may be too low to be of physiological significance. It is of interest to compare the distribution of transamidinase in the mammal with that of another enzyme involved in arginine metabolism, argininosuccinase, previously known to occur in mammals only in liver and kidney(10). Argininosuccinase has recently been found in this laboratory to occur in numerous other tissues of the body, such as brain, testis, spleen, pancreas, thymus, heart, and skeletal muscle. The significance of this distribution is not known at present.

Summary. Arginine-glycine transamidinase, one of the 2 enzymes concerned with the biosynthesis of creatine from amino acid precursors, has been found to occur in high concentration in mammalian pancreas. Homogenates of dog pancreas have approximately 5 times the transamidinase activity of kidney homogenates from the same animal, on a wet weight basis. The assay systems by which these tissues were compared included (a) canavanine-ornithine transamidination, made unidirectional by the addition of a carbonyl compound to trap canaline, and (b) arginine-hydroxylamine transamidination. It is suggested that the ability to catalyze these latter two reactions is a characteristic property of transamidinases. Chemical and enzymic syntheses of hydroxyguanidine are described.

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Effect of Cortisol on Gastric Ulcers of the Shay Rat. (23923)

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Since Shay *et al.*(1) published that gastric (rumenal) ulcers could be produced by ligating the pylorus of a fasting rat, numerous studies have appeared, using this technic, on the effect of various agents on formation of such ulcers and on the composition of the gastric juice which accumulates. It has been found that following adrenalectomy, rumenal ulcers do not develop(2,3) and acid and pepsin production by the mucosa is greatly diminished(4,5). Replacement therapy with corticoids, however, results in ulcer formation (3) coincidental with the return of normal gastric secretion(5). In the present communication, we report the influence of large doses of cortisol (COL) on gastric ulcers using the Shay rat preparation.

Materials and methods. A total of 98 female rats, of an average body weight of 160 g, of the Upjohn strain (originally from Sprague-Dawley) were divided into groups of 8-10 animals. Half of these served as controls (receiving cortisol vehicle), whereas the other half were treated with cortisol. At 9:00 a.m., 48 hours prior to operation, food was removed. Pylorus ligation was performed after this period of fasting according to Shay's procedure(1); however, in order to avoid any