Myotonic dystrophy: investigation of the proposed defect in guanidoacetic acid synthesis

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SUMMARY No significant abnormality was detected in plasma levels or urinary excretion of guanidoacetic acid (GAA) in patients with myotonic dystrophy. It therefore seems unlikely that there is defective synthesis of GAA in this disorder. It was confirmed that arginine-glycine amidinotransferase (AGA), the enzyme responsible for GAA synthesis, is present in renal cortical tissue, but no enzyme activity could be detected in a variety of other, more accessible tissues taken from healthy controls.

Myotonic dystrophy is a dominantly inherited disorder, the biochemical basis of which is unknown. It has been known for many years that a number of diseases with muscle wasting are characterized by an increased urinary excretion of creatine, which is believed to be secondary to the reduction in functioning muscle tissue (Milhorat, 1953). Various studies have shown that in myotonic dystrophy, however, urinary creatine levels are either normal or only slightly raised (Zierler, Folk, Magladery, and Lilienthal, 1949; Van Pilsum and Wolin, 1958). It has been suggested that the absence of excessive creatinuria in myotonic dystrophy may be due to defective synthesis of creatine (Zierler *et al.*, 1949), and Harvey (1969) has reported a reduction in arginine-glycine amidinotransferase (AGA; EC 2, 1, 4, 1) activity in renal cortical tissue from two patients with myotonic dystrophy. This enzyme is responsible for the formation of guanidoacetic acid (GAA), an intermediate in the synthesis of creatine, and is known to occur in human kidney cortex, pancreas and liver (Walker, 1963). However studies of GAA excretion in myotonic dystrophy have provided conflicting results. Van Pilsum and Wolin (1958) found that in myotonic dystrophy urinary GAA levels were elevated, whereas Harvey (1962) reported reduced levels in this condition.

The aim of the present study was to establish

whether there is a defect in the synthesis of GAA in myotonic dystrophy. The problem was approached in two ways: firstly, by comparing plasma and urinary GAA levels in patients and healthy controls and, secondly, because of the obvious difficulties of obtaining biopsy specimens of kidney, pancreas, and liver, by investigating whether AGA activity could be detected in more accessible tissues. If enzyme activity could be demonstrated in easily accessible tissues, it might then be possible to investigate in greater detail the reported decrease in enzyme activity in patients with myotonic dystrophy.

METHODS

SUBJECTS The diagnosis of myotonic dystrophy was established on the basis of clinical examination, electromyography, and in some cases, muscle histology. The patients varied from those who were minimally affected to those who were chair-ridden and severely incapacitated. Healthy individuals with no history of any neuromuscular disorder were used as controls.

GUANIDOACETIC ACID ESTIMATION The method used was a modification of that described by Van Pilsum, Martin, Kito, and Hess (1956). All estimations were carried out on fresh material. Blood was obtained by venepuncture after an overnight fast and collected in heparinized tubes. Plasma and red cells were separated by centrifugation at 500 *g* for 10 min. It was confirmed that GAA is absent from erythrocytes (Sandberg, Hecht and Tyler, 1953). Twenty-four

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hour urine specimens were collected with a few small crystals of thymol added as a preservative. It was established that the presence of thymol did not affect the assay.

On examination of the assay method it was found that when a known amount of GAA was added to the urine after deproteinization, incomplete recovery was obtained. When GAA standards were prepared in diluted deproteinized urine, the same percentage recovery was obtained at each concentration of GAA. A different percentage recovery was obtained with different urine samples. Therefore a correction was made to the GAA level observed in each urine sample after calculating the percentage recovery of a known amount of added GAA. Complete recovery was obtained when GAA was added to plasma.

ARGININE-GLYCINE AMIDINOTRANSFERASE ASSAY The method used was a modification of that described by Walker (1960). Tissues were homogenized in 0.1 M phosphate buffer pH 7.4 containing 0.001 M EDTA. Erythrocytes, leucocytes, cultured cells, and urine sediment were suspended in phosphate buffer and sonicated before assay. Urine sediment and supernatant were prepared by centrifugation of a 24-hour specimen (collected without preservative) at 8,000 *g* for 20 min at 4° C. 250 ml. of the supernatant were dialyzed against water at 4° C for three days, and then freeze dried. The residue was dissolved in a small volume of phosphate buffer and assayed. The assay mixture for all determinations contained 0.2 ml. sample, 0.05 ml. 1 M phosphate buffer pH 7.4, 0.05 ml. 1 M L-arginine hydrochloride, and 0.1 ml. 2 M hydroxylamine hydrochloride in 2 M potassium hydroxide. After incubation at 37° C, the reaction was terminated by the addition of 0.3 ml. 20% trichloroacetic acid. After centrifugation a 0.5 ml. portion of the supernatant was mixed with 0.5 ml. 1 M phosphate buffer pH 7.0 and 0.1 ml. acetone and then 0.1 ml. 1% ammonium disodium pentacyanoferrate was added. After 20 min extinction was read at 480 nm. In each assay two blanks were used:

in one, the homogenate was replaced by buffer and in the other, arginine was replaced by water. All samples were read against a reference sample in which both homogenate and substrate were omitted. In an attempt to detect low levels of enzyme activity, the reaction mixture was incubated at 37° C for periods of up to five hours. Experiments using human kidney cortex showed that the rate of enzyme action was linear up to five hours.

Total protein determinations were carried out according to the method of Papadopoulos, Hess, O'Doherty, and McLane (1959).

RESULTS

Fasting plasma GAA levels were determined in six patients with myotonic dystrophy and in seven controls. The levels in all cases were close to the limit of sensitivity of the assay, being less than 0.35 mg/100 ml. There was no apparent difference between the values obtained in the patients and in the controls.

The urinary excretion of GAA in healthy individuals was unrelated to age but was significantly greater in females than in males when expressed as mg of GAA excreted/24 hr/kg body weight ($P < 0.02$). There was greater variability in the results from females than from males. For these reasons the values for patients and controls of the same sex were compared (Table 1). The results show that there is no significant difference in the urinary excretion of GAA between patients with myotonic dystrophy and controls. There was greater variability in the values for affected males than for control males, but this variability did not appear to be related to the severity of the disease.

The presence of AGA activity in renal cortical tissue was confirmed. Assay of enzyme activity in necropsy and biopsy material from individuals with no evidence of renal disease and no neuromuscular disorder gave a value for one biopsy specimen within the range observed for six necropsy specimens. However, enzyme activity in biopsy specimens from three kidneys with calculi was significantly greater than in healthy kidneys (Table 2).

AGA activity could not be detected in any of the following tissues obtained from healthy controls: plasma, erythrocytes, leucocytes (uncultured and cultured for 72 hours with phytohaemagglutinin), whole skin and cultured skin

TABLE 1
URINARY EXCRETION OF GUANIDOACETIC ACID

	Controls		Myotonic dystrophy	
	Males	Females	Males	Females
Subjects (no.)	13	13	7	4
Age range (yr)	22-45	23-57	29-58	20-31
GAA (mg/24 hr)	43.8 ± 9.6	52.5 ± 25.2	41.1 ± 19.5	42.3 ± 9.9
GAA (mg/24 hr/body wt)	0.61 ± 0.13	0.93 ± 0.41	0.67 ± 0.35	0.86 ± 0.27

Results expressed as mean ± standard deviation.

TABLE 2
ARGININE-GLYCINE AMIDINOTRANSFERASE ACTIVITY
IN KIDNEY CORTEX

Source of material	Condition of kidney	Specimens (no.)	Age range (yr)	AGA activity (n-mole/min/100 mg protein)
Necropsy	No evidence of disease	6	40-69	167.0 ± 42.7
Biopsy	Healthy	1	40	128.5
Biopsy	Calculi	3	40-60	340.8 ± 12.2

Results expressed as mean ± standard deviation.

fibroblasts, muscle tissue and cultured myoblasts, cerebrospinal fluid, and sediment and concentrated supernatant from 24 hour specimens of urine.

Since aspirin is known to cause shedding of renal tubular cells into the urine (Scott, Denman, and Dorling, 1963; Prescott, 1965), an attempt was made to produce measurable enzyme activity in urinary sediment by administering aspirin to a healthy male subject and collecting serial urine samples at 16 hour intervals up to 64 hours. Even after administration of 3 g aspirin, only very low enzyme activity was detectable in the urine sediment, this activity being confined to the sample collected between 16 and 32 hours after treatment. Because this enzyme activity was so low in a healthy individual it did not seem justified to extend this investigation to patients with myotonic dystrophy.

DISCUSSION

Since no significant abnormality was detected in plasma levels or urinary excretion of GAA in patients with myotonic dystrophy, it seems unlikely that there is a defect in GAA synthesis associated with this disease. AGA activity could not be detected in a number of easily accessible tissues from healthy controls. Thus if the reported decrease in AGA activity in myotonic dystrophy (Harvey, 1969) is to be further investigated there is no alternative but to study kidney,

liver, or pancreatic tissues. However, it seems unjustified to subject patients to the dangers of taking biopsies from such tissues, since the present study gave no evidence for suspecting any abnormality in the synthesis of GAA in myotonic dystrophy.

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