An Investigation Into The Biochemical Genetics of β-Aminoisobutyric Aciduria*

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INTRODUCTION

β-AMINOISOBUTYRIC ACID (BAIB) is a non-protein amino acid found in human urine in widely varying concentrations from one person to another. Several studies have demonstrated that this variation is largely under genetic control, with most of the variability being due to differences at a single locus (Harris 1953; Gartler, Firschein and Kraus 1957). The present report is concerned with the question of the mode of action by which the genetic differences lead to the observed variability in urinary BAIB excretion rates.

Crumpler, Dent, Harris, and Westall (1951), in their original paper describing β-aminoisobutyric aciduria, suggested that the underlying mechanism in this condition was a genetically controlled renal tubular defect. The supporting evidence for this hypothesis was the fact that they did not find gross differences between the blood levels of BAIB in individuals with β-aminoisobutyric aciduria (high excretors) and individuals excreting only small amounts of BAIB (low excretors). They argued that if a block in the intermediary metabolism of BAIB, rather than a renal defect, were responsible for this amino-aciduria, the plasma level of BAIB would be markedly elevated in high excretors.

For substances which are normally reabsorbed from the glomerular filtrate with a high degree of efficiency (e.g., protein occurring amino acids), this argument is valid. However, when a substance is normally reabsorbed with a low efficiency, a block in intermediary metabolism will not lead to its accumulation in the blood (e.g. alcaptonuria), and in such instances, comparative blood levels for the substance might not be critical. Nothing is known about the normal absorption rate for BAIB. Furthermore, though the immediate cause of an aminoaciduria may be renal, the primary genetic lesion may be one of intermediary metabolism which indirectly brings about renal damage and aminoaciduria (e.g., galactosemia and Wilson's disease).

In view of these uncertainties, it appears that the question of the underlying mechanism in β-aminoisobutyric aciduria is unanswered, and the following studies were undertaken to investigate this problem more fully.

MATERIALS AND METHODS

BAIB and related substances (thymine, DHT and BUIB)[1] were administered to normal high and low excretor subjects (Table 1). BAIB, thymine, and DHT were

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[1] DHT = dihydrothymine. BUIB = β-ureidoisobutyric acid.

TABLE 1. AGE, SEX, WEIGHT, AND FASTING BAIB EXCRETION VALUES FOR THE NORMAL SUBJECTS USED IN THESE EXPERIMENTS

Subject	Age	Sex	Weight	Fasting Urinary BAIB Excretion Mg. BAIB in 6 hours*
Low Excretors				
SG	34	M	69 Kg.	5.3
LL	22	M	60	2.5
BV	27	M	67	3.3
PC	21	F	52	3.1
JS	20	F	63	6.0
BW	40	F	56	3.4
High Excretors				
HW	20	M	60	45.7
JA	24	M	65	44.1
ML	32	F	45	34.3
RG	21	F	51	55.4

* Based on at least two determinations taken on different days.

obtained from the California Foundation for Biochemical Research. BUIB was prepared in the laboratory according to the method of Fink, McGaughey, Cline and Fink (1956). At appropriate times, blood and urine specimens were taken and analyzed for BAIB and, in some cases, for thymine, DHT, and BUIB. The data thus obtained permitted (1) analysis of the normal renal reabsorptive values for BAIB, (2) comparison of renal function with regard to BAIB in high and low excretors, and (3) comparison of the metabolic pathway involving BAIB in high and low excretors.

Urine specimens were either analyzed the day of collection or stored in a frozen state until analyzed. Serum was treated with four volumes of ethanol, the precipitate removed by centrifugation, and the ethanolic extract used directly for analysis.

BAIB determinations were carried out by high voltage electrophoresis on paper (Gartler 1959). DHT and BUIB determinations were made by the methods of Fink, McGaughey, Cline and Fink (1956). Thymine was detected by examining paper chromatograms under ultra violet light.

RESULTS

Renal Excretion of BAIB in High and Low Excretors

It has not been possible to detect BAIB in the fasting serum of any of the subjects investigated, even though the methods used were sensitive to less than 0.1 mg. per cent of serum BAIB. The average BAIB urinary excretion rates reported in Table 1 serve to class BAIB as a low threshold substance or one that is normally reabsorbed with a low degree of efficiency. At such low blood concentrations (less than 0.1 mg. per cent) the average minimal renal clearance for the high excretors approaches the glomerular filtration rate, while for the low excretors, the average minimal renal clearance would be many-fold greater than that for comparable substances, such as other amino acids.

Feeding experiments were undertaken to increase the serum BAIB to measurable

TABLE 2. RENAL CLEARANCES OF BAIB IN LOW AND HIGH EXCRETORS AFTER THYMINE ADMINISTRATION

Subjects	Mg. Thymine Administered	Mg. BAIB Ml. Serum	Mg. BAIB Excreted/ minute	Renal* Clearance
Low Excretors				
PC	500	0.0025	0.39	156.0
PC	1500	0.0058	1.47	253.4
LL	500	0.0050	0.80	160.0
SG	1500	0.0060	1.43	238.3
JS	1500	0.0045	0.94	208.9
BV	500	0.0020	0.35	175.0
High Excretors				
ML	500	0.0031	0.50	161.3
ML	1500	0.0060	1.47	245.0
RG	500	0.0080	0.78	97.5
RG	1500	0.0100	1.45	145.0
HW	500	0.0045	0.60	133.3

* Ml. serum cleared/minute.

levels. Thymine, a highly effective precursor of BAIB in man (Awapara and Shullenberger (1957), Gartler (1959)) was orally administered. The BAIB excreted after thymine administration is the L form, which is excreted in β-aminoisobutyric aciduria (Gartler, unpublished data). Blood samples were obtained two hours after thymine administration and urine specimens were collected fifteen minutes before and fifteen minutes after the drawing of blood samples. Table 2 lists the results of these experiments. As can be seen, there is no evidence of tubular reabsorption of BAIB at these blood levels (0.2 mg. per cent–1.0 mg. per cent). The renal clearances are all very high, and with one exception, exceed the normal glomerular filtration rate. Of particular interest are those instances where increasing dosages of thymine were administered to the same individuals (P. C., M. L., and R. G.). In all cases, the renal clearances were increased to such high levels as to indicate the possibility of active tubular excretion of BAIB.

METABOLISM AND EXCRETION OF ADMINISTERED DL-BAIB IN HIGH AND LOW EXCRETORS

In view of the results of the preceding experiments, I decided to investigate the effects of the administration of BAIB, though the only form of this substance available was the racemic mixture. Table 3 presents the results of the experiments involving the oral administration of DL-BAIB to high and low excretors of BAIB. The low excretors retained almost all of the administered BAIB, whereas the high excretors excreted approximately 50 per cent of the administered material.

To circumvent the possible complications of variable intestinal absorption, BAIB was administered intravenously at two dosages to one low and one high excretor subject. Urine specimens were collected hourly, and it was found that normal BAIB excretion rates were resumed approximately one hour after BAIB injection. The results of these experiments are given in Table 4. They support the conclusions of the experiments involving the oral administration of BAIB. Blood specimens drawn

TABLE 3. URINARY EXCRETION OF BAIB AFTER THE ORAL ADMINISTRATION OF 50 MG. DL-BAIB

	Mg. BAIB excreted in 6 hours (Corrected for fasting BAIB level)
Low Excretors	
SG	2.4
BW	8.3
BV	3.5
High Excretors	
ML	37.2
JA	24.4
HW	23.1

TABLE 4. URINARY EXCRETION OF BAIB FOLLOWING THE INTRA-VENOUS INJECTION OF DL-BAIB

Subject	Mg. DL-BAIB Injected	Mg. Injected BAIB* Excreted in the Urine
Low Excretor (S. G.)	10	0.7
	20	2.9
High Excretor (H. W.)	10	7.2
	20	9.0

* Over 90 per cent of the recovered BAIB was excreted in the first hour following BAIB injection.

from the low excretor in the 20 mg. experiment at three minutes and 90 minutes after BAIB injection gave values of 0.47 and less than 0.10 mg. per cent, respectively. The 2.9 mg. excreted in the urine accounts for only a small fraction of the reduction in serum BAIB. It seems clear, therefore, that the major portion of BAIB in the low excretor is cleared by a different mechanism from that used by the high excretor. The fact that a considerable amount of the injected BAIB was retained by the high excretor in the 20 mg. experiment indicates that the high excretor may also use more than one mechanism for BAIB clearance.

An interesting sideline of the DL-BAIB experiment is that the low excretors apparently do not discriminate between the D and L forms of BAIB. For most amino acids, this would be unusual, but in the case of BAIB, which is not a structural substance, the absence of stereoisomeric specificity may not be critical.

DISCUSSION

There are at least three possible mechanisms of gene action which can lead to a genetically determined aminoaciduria; (1) renal defect, (2) block in precursor metabolism, and (3) differential utilization.

The data presented in the first section of this paper give no evidence for any significant difference in renal function between low and high excretors. In fact, there is no evidence for tubular reabsorption of BAIB at the serum levels studied in either high or low excretors, which argues strongly against the renal hypothesis of β-aminoisobutyric aciduria.

The possibility of a block in precursor metabolism (thymine $\rightleftarrows$ DHT $\rightleftarrows$ BUIB $\rightarrow$ BAIB) was investigated in a previous study (Gartler 1959), and was excluded as the underlying factor in β-aminoisobutyric aciduria.

In contrast to the preceding negative evidence, the experiments involving BAIB

administration have demonstrated a striking difference between low and high excretors in BAIB utilization. Whereas low excretors retain nearly all of the administered load, high excretors eliminate 50 per cent or more of it. Furthermore, the disappearance of the injected BAIB from the blood of the low excretor would make it seem most likely that we are dealing with differential metabolism of BAIB. Just what the difference is between low and high excretors in their utilization of BAIB is not at all clear. Conversion of BAIB to BUIB by low excretors could explain this difference, but there is no evidence for significant *in vivo* reversibility of the BUIB → BAIB step. Fink, McGaughey, Cline, and Fink (1956) found no BUIB after BAIB administration in the rat, nor was any BUIB detectable in either blood or urine specimens after relatively large dosages of BAIB (250 mg.) in these experiments. Kupiecki and Coon (1957) have shown that, in the pig, BAIB is involved in the following reaction:

β-aminoisobutyrate + α-ketoglutarate ⇄
glutamate + methylmalonate semialdehyde.

This is a possible mechanism for the differential metabolism of BAIB, but thus far we have not been able to demonstrate this reaction in human tissues. Other possibilities exist (e.g., BAIB → isobutyric acid) but as yet, no definitive evidence has been obtained on this point.

SUMMARY AND CONCLUSIONS

An investigation into the biochemical genetics of β-aminoisobutyric aciduria has indicated that the most likely explanation of the difference between high and low excretors is differential metabolism of BAIB. Studies have shown that no significant difference in renal function exists between low and high excretors, nor is there any metabolic block in the pathway leading to BAIB formation. However, there is a marked difference between low and high excretors in their utilization of administered BAIB. The exact nature of this last step is not known, but various possibilities are discussed.

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