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CYSTATHIONINE β-SYNTHASE DEFICIENCY: METABOLIC ASPECTS

S. Harvey Mudd
National Institute of Mental Health, Bethesda, MD,

HOMOCYSTEINE METABOLISM: FROM BASIC SCIENCE TO CLINICAL MEDICINE

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MEDFORD, MA
USA

S. Harvey Mudd
National Institute of Mental Health
Bethesda, MD, USA

11. CYSTATHIONINE β-SYNTHASE DEFICIENCY: METABOLIC ASPECTS

S. Harvey Mudd

Introduction

Cystathionine β-synthase (CBS) deficiency was first demonstrated in 1964 in an eight-year-old mentally retarded girl with bilaterally dislocated optic lenses who excreted abnormally elevated amounts of homocystine in her urine [1]. Patients with similar metabolic abnormalities and clinical findings had first been discovered 2 years earlier by Carson and her colleagues during a survey of mentally backward children in Northern Ireland [2]. CBS deficiency has proven to be the most frequently encountered of the human genetic diseases causing homocystinuria and severe hyperhomocyst(e)inemia. Worldwide, it is detected with a frequency of about 1:344,000 by screening programs of the newborn, but this is undoubtedly an underestimate because some individuals are being missed [3]. This chapter will briefly focus on the major clinical manifestations and metabolic aspects of CBS deficiency.

Major Clinical Features

Clinically, the most prominent features of CBS deficiency are mental retardation, dislocation of the optic lenses, a tendency toward early thromboembolic events, and osteoporosis and other bony abnormalities. An international survey published in 1985 [4] presented data on 629 CBS-deficient patients. Among those so classified, some 44% were judged to be B_6-responsive; that is, when given high doses of pyridoxine they underwent prompt and marked decreases in plasma homocyst(e)ine and methionine concentrations. An equal proportion were judged nonresponsive, with little or no decreases in the abnormally elevated concentrations of these amino acids following pyridoxine administration. Although this crude classification is undoubtedly an oversimplification, the results clearly showed that in the absence of specific treatment, as a group the B_6-nonresponsive patients were more severely affected clinically than were the B_6-responsive patients. The median IQ for nonresponders was 56; for responders, 78. Among untreated patients, optic lens dislocation had occurred in 50% of nonresponders by age six years, a clinically apparent thromboembolic event had occurred in 25% of patients by age 15 years, and spinal osteoporosis was detected in 50% by age 12. By contrast, the corresponding ages for untreated B_6-responders were 10, 20, and 20 years.

Metabolic Aspects

The primary abnormality in CBS-deficient patients is a failure to covert homocysteine to cystathionine, a process that normally must occur chiefly in the liver. Mansoor and colleagues [5] have shown that in normal plasma, only 2% of the homocysteine-derived moieties exist as homocysteine itself; 16% occur as free disulfides (homocystine, homocysteine-cysteine mixed disulfide, other mixed disulfides); and 82% are bound to protein as mixed disulfides of homocysteine and protein cysteine [5]. Comparable measurements by modern methods have not been reported for intracellular homocysteine-derived moieties in CBS-deficient patients. Clearly there is some export of the abnormally accumulated homocysteine so that the total plasma homocyst(e)ine (tHcy) rises dramatically. The greatest relative rise occurs in homocysteine, with lesser rises in free disulfides and the protein-bound mixed disulfide (up to 400-fold, 90-fold, and 20-fold above normal, respectively) [6]. Plasma methionine also rises in most CBS-deficient patients, presumably due to enhanced methylation of abnormally elevated homocysteine. The source(s) of the methyl group for such methylation is (are) uncertain. The availability of betaine must be limiting under normal dietary conditions because administration of betaine usually results in further decreases in tHcy. N^5-Methyltetrahydrofolate would be expected to provide an alternative supply of methyls (see below).

Additional compounds that would be expected to accumulate abnormally in cells of CBS-deficient pa-

tients include S-adenosylhomocysteine (AdoHcy) (if adenosine is available) and S-adenosylmethionine (AdoMet). The former is expected to accumulate because the equilibrium of AdoHcy hydrolase favors AdoHcy formation, and the latter because of the elevated methionine concentration in the presence of intact methionine adenosyltransferase that (in the liver) is normally operating far below saturation with respect to that substrate. Neither of these compounds has been measured in liver or other tissue of CBS-deficient patients by use of methods that are now known to be necessary to avoid rapid changes after tissue is removed from the body. Indirect evidence for an abnormal elevation of AdoHcy is provided by the fact that Perry and coworkers [7,8] found unusual amounts of this compound and of 5-amino-4-imidazolecarboxamide-5′-S-homocysteinylriboside, a compound structurally (and presumably metabolically) closely related to AdoHcy, in the urines of their CBS-deficient patients.

The intracellular AdoMet/AdoHcy ratio, often termed the "methylation index," will determine the relative activities of most AdoMet-dependent methyltransferases [9]. It can be inferred that this ratio may be relatively undisturbed in CBS-deficient patients by the fact that they seem not to suffer from the neurologic abnormalities associated with defective myelination that are common in patients with methylenetetrahydrofolate reductase (MTHFR) deficiency [10] who, because of their low, or low normal, methionine and elevated tHcy concentrations, may be expected have low AdoMet/AdoHcy ratios. Jencks and Matthews stated that the physiologic activity of MTHFR "must . . . be determined by competition between AdoMet and AdoHcy for a common ligand-binding site and would therefore be expected to be very sensitive to the AdoMet/AdoHcy ratio" [11]. The inferred normality of this ratio in CBS-deficient patients would mean that there is no interference with the biosynthetic pathway to N^5-methyltetrahydrofolate, and that there should be sufficient amounts of this compound to support methylation of the abnormally elevated homocysteine. The increased input of methyl groups through homocysteine methylation raises the problem of how these methyl groups are to be disposed of. If utilization for homocysteine methylation keeps N^5-methyltetrahydrofolate at a low concentration, glycine methyltransferase will be active and methyl groups will flow to sarcosine as a step in their ultimate oxidation. It would thus be of interest to assess quantitatively the rates of homocysteine methylation and sarcosine formation in CBS-deficient patients.

The metabolic consequences of CBS deficiency might be expected to include abnormally low rates of synthesis of compounds distal to the enzyme block, including cystathionine and cysteine. Because a relatively small residual activity of CBS may support a substantial flux of homocysteine into cystathionine (see below), the extent of the abnormalities in cystathionine and cysteine in a given patient will probably depend upon how much residual CBS activity is present. Cystathionine is present in normal human brain at high concentrations [12], but was present at most only in trace amounts in brains obtained at postmortem examination of three homocystinuric individuals [13,14]. In the absence of data on the B_6-responsiveness of these patients, it is uncertain whether or not they had any residual CBS activity. Reliable assays of tissue cysteine concentrations have not been reported, but Mansoor et al. [6] found that in seven patients with homocystinuria and hypermethioninemia consistent with CBS deficiency (but not specified with respect to B_6-responsiveness), plasma concentrations of total cyst(e)ine ranged from 52 to 167 μM, below the mean normal value of approximately 250 μM [6].

The Mechanism of B_6-responsiveness

As discussed above, a major determinant of the clinical prognosis for CBS-deficient patients is whether or not they are B_6-responsive. In considering possible mechanisms of response it is important to realize that although B_6 administration may bring about marked drops in plasma tHcy and methionine in responsive patients together with rises in abnormally low plasma cyst(e)ine concentrations, these patients are not restored to biochemical normalcy. They usually continue to have some elevation of homocystine in plasma and urine, and respond abnormally to a methionine load as judged by the extent and/or duration of elevations of plasma methionine and of plasma and urinary homocystine [15,16].

In principle, the B_6-induced response could be due either to stimulation of an alternative pathway for catabolism of methionine or homocysteine, or to stimulation of residual CBS activity. No evidence has been reported indicating stimulation of an alternative pathway, and indeed there is a possibility that the methionine transamination pathway, which is the major known alternative route for methionine degradation, is inhibited in CBS-deficient patients [17]. Furthermore, the genetic evidence now available proves that B_6-responsiveness or nonresponsiveness is determined by some property of the mutant CBS itself. This was first indicated by the fact that B_6-

TABLE 11-1. Cystathionine β-synthase activities in livers of cysathionine β-synthase-deficient patients

B₆-responsive		B₆-nonresponsive		
B₆ administration				
no	yes	no	yes	
Liver CBS activity, % of mean control*				Citation
12.3**	—			35
30.5**	—	0	—	36
2.0	3.0			15
1.2	4.6			
3.7	16.5	0	0	37
9.4	11.7	0	0	
5.9	22.6			
5.6	24.7			
15.3**	55.1			
7.1	6.7			38
6.2	10.7			
4.0	8.4			

* All in vitro assays with pyridoxal-5'-phosphate added.
** Patient with less marked homocystinuria: genetic status uncertain.

responsiveness or nonresponsiveness is the same for all CBS-deficient sibs within a given sibship [4], suggesting that the same (or a closely linked) genetic mutation that makes an individual CBS-deficient determines whether he will be B₆-responsive. More recent studies of mutations in the CBS gene have firmly supported this conclusion. Striking examples include the findings that "the 'Celtic' mutation G307S appears to be incompatible with B₆ responsiveness whether it is present in one or two copies in the patient" [18], and that the prevalent mutation I278T is associated in homozygotes with B₆ responsiveness and, in compound heterozygotes, with some degree of B₆ responsiveness [19].

A few assays of CBS activity in livers of CBS-deficient patients were performed before the discovery that this activity is expressed in normal cultured skin fibroblasts later obviated the need for studies of liver for diagnostic purposes. Those carried out with sufficient sensitivity to detect relatively small residual CBS activities are summarized in table 11-1. Each B₆-responsive patient had detected residual activity, and treatment of the patient with therapeutic doses of B₆ almost invariably led to at least some increase in the hepatic activity. Pyridoxal 5'-phosphate was added in vitro for these assays. This fact, in addition to other available evidence [3], makes it clear that in no instance was the deficit in activity overcome merely

by the addition of cofactor, as would be expected were these simply K_m mutations. By contrast, no B₆-nonresponsive patient, whether or not given B₆, had detected hepatic activity.*

The physiologic importance of the relatively small activities of CBS in the B₆-responsive patients has been indicated by measurements of the abilities of these patients to convert the sulfur of an oral load of methionine to inorganic sulfate, and by determination by nitrogen balance studies of their capacities to form cysteine from methionine. The normal human has the capacity to convert methionine sulfur to inorganic sulfate at a rate of at least 84 millimole/day. In their basal states, B₆-responsive CBS-deficient patients had a mean capacity of 8.3 millimole/day, and this capacity was increased by B₆-treatment to 16.1 millimole/day. Although each of these rates is small compared to normal, they are sizable compared to the normal methionine intake of some 8–12 millimole/day [15]. Finally, in contrast to normal humans, B₆-nonresponsive CBS-deficient patients do not maintain

* The results of assays of CBS activities in cultured skin fibroblasts generally support the suggestion that B₆-responsive patients have small residual activities of CBS, whereas B₆-nonresponsive patients do not [33]. However, experience has shown that such results are fallible, at least in the sense that individuals who must have CBS activity in their liver may fail to manifest such activity in cultured fibroblasts. See, for example, de Franchis et al. [34].

nitrogen balance when given methionine-adequate, cystine-free diets [20,21]. However, B_6-responsive patients, even on normal B_6 intakes, did remain in nitrogen balance on such diets. Titration of the amount of cystine required to maintain balance in a nonresponsive patient indicated that the B_6-responders must be synthesizing each day at least 2.5–4.0 millimole of cysteine, a minimal value that is sizable compared to the normal methionine intake [21] and that, if stimulated by no more than two- to threefold, would become sufficient to metabolize such an intake.

Are the Vascular-damaging Effects of Hyperhomocysteinemia Somehow Partially Mitigated in CBS-deficient Patients and in Heterozygotes for CBS Deficiency?

The results of long-term treatment of CBS-deficient patients are now beginning to be reported [22–24]. B_6-nonresponsive patients have been free of thromboembolic events, both those in Australia treated with betaine, B_6, folate, and B_{12} [22,23], and those in Ireland given low-methionine diets (E Naughten, personal communication). This is surprising in view of the fact that, for the Australian patients, medications were given in doses that maintained plasma free homocyst(e)ine at "usually . . . less that 60 μM" [22], so that plasma tHcy may have been at least twice this value [6]—i.e., at least 120 μM. Compared with the levels of hyperhomocyst(e)inemia reported in the abstracts and papers for this meeting to confer significant relative risks of thromboembolic phenomena, 120 μM is extremely high. Why have these patients been free of thromboembolism?

The same question arises with regard to heterozygotes for CBS deficiency. Such individuals are not overrepresented among individuals with premature vascular disease [25,26], and are at little, if any, increased relative risk for such disease [27,28]. Could it be that the vascular-damaging effects of hyperhomocyst(e)inemia are somehow mitigated in individuals with partial or severe lack of CBS activity? Perhaps methionine acts as a competitive structural inhibitor of this damage, and the elevated concentrations of this amino acid in CBS-deficient individuals, in contrast to its normal or low concentrations in those with homocysteine methylation difficulties, is protective. Other differences exist between individuals with impaired CBS and impaired homocysteine methylation—the former have higher concentrations of AdoMet and lower concentrations of cystathionine and, perhaps, cysteine; and those with milder impair-

ments are likely to have hyperhomocyst(e)inemia only following intake of methionine (i.e., at times when the methionine concentration is also high), rather than fasting hyperhomocyst(e)inemia [29,30]. Again, if the transsulfuration pathway is normally not operative in endothelial cells and vascular tissues (a point upon which present evidence conflicts [31,32]), one might expect the pathophysiologic effects of a given extent of hyperhomocyst(e)inemia to be less severe with impairments of CBS than of homocysteine methylation (JD Finkelstein, personal communication). These possible mitigating effects are not mutually exclusive. They may merit being borne in mind as work progresses on hyperhomocyst(e)inemia and its role in cardiovascular disease. Perhaps nature's experiments with CBS-deficiency have lessons to teach us worthy of our attention.

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