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Hyperhomocysteinemia and function of the endoplasmic reticulum

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Commentary

Study of hyperhomocysteinemia (HHcy) began with the desire to understand the complex phenotype associated with rare monogenic mutations that perturb homocysteine (Hcy) and methionine metabolism. More recently, epidemiological surveys have suggested a positive link between serum Hcy levels and the development of ischemic heart disease and stroke (1). While the correlative observations fall short of implicating HHcy directly in the pathophysiology of these common disorders, the possibility that HHcy may be more than a marker for some underlying cause of atherosclerosis and thrombosis has energized research in the field. This issue of the JCI contains an article drawing attention to a potential link between HHcy, perturbation in function of the endoplasmic reticulum (ER), and alterations in lipid and cholesterol metabolism (2). Here I wish to provide a perspective on these interesting observations. Severe monogenic HHcy (also referred to as homocysteinuria) is commonly caused by mutations in the cystathionine β-synthase (CBS) gene that block the use of Hcy in the biosynthesis of cysteine. Less common are mutations in the methylenetetrahydrofolate reductase (MTHFR) gene that indirectly block intracellular conversion of Hcy to methionine. Both classes of mutation elevate intracellular and circulating Hcy levels and increase the propensity for large-vessel venous and arterial thrombosis. Other manifestations are specific to mutations in each gene. For example, marfanoid features, osteoporosis, and dislocation of the lens […]

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Study of hyperhomocysteinemia (HHcy) began with the desire to understand the complex phenotype associated with rare monogenic mutations that perturb homocysteine (Hcy) and methionine metabolism. More recently, epidemiological surveys have suggested a positive link between serum Hcy levels and the development of ischemic heart disease and stroke (1). While the correlative observations fall short of implicating HHcy directly in the pathophysiology of these common disorders, the possibility that HHcy may be more than a marker for some underlying cause of atherosclerosis and thrombosis has energized research in the field. This issue of the *JCI* contains an article drawing attention to a potential link between HHcy, perturbation in function of the endoplasmic reticulum (ER), and alterations in lipid and cholesterol metabolism (2). Here I wish to provide a perspective on these interesting observations.

Severe monogenic HHcy (also referred to as homocysteinuria) is commonly caused by mutations in the cystathionine β-synthase (*CBS*) gene that block the use of Hcy in the biosynthesis of cysteine. Less common are mutations in the methylenetetrahydrofolate reductase (*MTHFR*) gene that indirectly block intracellular conversion of Hcy to methionine. Both classes of mutation elevate intracellular and circulating Hcy levels and increase the propensity for large-vessel venous and arterial thrombosis. Other manifestations are specific to mutations in each gene. For example, marfanoid features, osteoporosis, and dislocation of the lens are found in patients with CBS deficiency, whereas late-onset psychiatric symptoms and neurological dysfunction appear to be more common in patients lacking MTHFR (3). The link between Hcy levels and vascular disease in the general population and the finding that thrombosis is a feature common to HHcy of different causes have directed attention to this clinical manifestation.

HHcy is not associated with abnormalities in serum lipid levels, nor is it believed to affect directly the activity of circulating coagulation factors or platelets. Therefore, much of the research in the field has focused on the interaction of Hcy with vascular tissue components. Abnormal vascular responses can be induced in otherwise healthy cynomolgus monkeys by dietary manipulations that increase circulating Hcy levels (4). The normal vasodilator properties of endothelium are inhibited by Hcy, and this inhibition is subject to modulation by nitric oxide. In addition, Hcy has been found to impair the endothelial cell's response to oxidative stress (5). However, the molecular mechanisms by which Hcy contributes to oxidative stress are not known. Furthermore, it is argued that if nonenzymatic oxidation reactions underlie the toxicity of Hcy, the related amino acid cysteine, which is present at much higher concentration, should contribute more substantially to oxidative stress; yet this is clearly not the case. These considerations have left the door open to the possibility that the toxicity of Hcy has other components.

Several groups have recently taken an unbiased approach to identifying cellular processes impacted by HHcy. They looked for genes induced in cells cultured in vitro in media with high concentrations of Hcy. Surprisingly, Hcy was found to activate the expression of genes known to be under the control of signaling pathways that respond to load on the ER. These include the *BiP/GRP78* gene, encoding an ER chaperone (6); *CHOP/GADD153*, encoding a transcription factor implicated in cellular responses to ER stress (7, 8); and *HERP*, encoding a protein that may be involved in degradation of malfolded ER proteins (9). The cellular response to ER load/stress includes an evolutionarily conserved transcriptional adaptation by which cells adjust the biosynthesis of proteins involved in ER function and secretion to the physiological demand made on the organelle. It is known as the unfolded protein response (UPR) and is effected by several partially redundant signaling pathways that monitor the folding environment in the lumen of the ER and transduce signals across the membrane to the cytoplasm and nucleus (10, 11). The UPR not only responds to increased physiological load on the ER but also is activated by perturbations in function of the organelle. In fact, the ability to specifically perturb ER function by toxins (e.g., tunicamycin, which inhibits N-linked protein glycosylation) has significantly simplified study of the UPR.

The paper by Werstuck et al. in this issue of the *JCI* adds several important components to the analysis of the link between the UPR and HHcy (2). It provides strong evidence that the induction of gene expression in Hcy-treated cells is due to activation of the UPR and not some parallel signaling pathway that happens to share the same target genes: Excess, free lumenal BiP/GRP78 has been shown to attenuate the UPR (12). One mechanism that contributes to this attenuation is BiP binding to the lumenal domains of the apical signaling proteins of the UPR, IRE1 and PERK, inhibiting their activation (13). The ability of BiP/GRP78 overexpression to block activation of target genes by Hcy indicates that some aspect of ER function is perturbed in Hcy-treated cells (2).

Earlier papers describing the induction of the UPR in Hcy-treated cells used very high (1 mM range) concentrations of the amino acid; lower concentrations

had only modest effects on gene expression in cultured cells. Given that the concentration of Hcy measured in the patients' serum rarely exceeds the 100-μM range, the physiological significance of the induction of the UPR by the high concentrations used in the in vitro experiments was questioned. Werstuck and colleagues (2) deal with this problem by showing that diet-induced HHcy causes marked elevation of CHOP/GADD153 expression in the livers of mice, indicating that the activation of the UPR in Hcy-treated cells is unlikely to be a tissue-culture artifact (2). At this point it is therefore legitimate to consider the possible role that ER dysfunction and the UPR may have in the pathophysiology of HHcy.

Diet-induced HHcy is associated with the induction of a proinflammatory state in the endothelium of large arteries. One of the striking features associated with this inflammation is the activation of the transcription factor NF-κB in cells of the affected endothelium — activation that is thought to control downstream inflammatory processes (14). Pahl and Baeuerle have reported the activation of NF-κB in cells experiencing ER overload (15). This suggests one mechanism for a direct link between the effects of Hcy on ER function and the proinflammatory state in the blood vessels. ER stress-mediated activation of IRE1 also leads to phosphorylation and activation of stress-activated protein kinases of the JNK family, which may contribute further to inflammation and even cell death (16).

Over ten years ago Lentz and colleagues noted that Hcy can inhibit the biosynthesis of proteins normally secreted by endothelial cells and that this inhibition is associated with ER retention of the proteins and their ultimate degradation (17, 18). Given that the ER has a tight quality control system that prevents the export of imperfectly folded proteins, it is tempting to speculate that the activation of the UPR and the retention of secreted proteins are both consequences of perturbed ER function induced by HHcy. Defective ER function may alter the expression of cell surface proteins or the secretion of proteins with local anti-inflammatory and antithrombotic properties. The basis for the impairment in ER function by Hcy is not known, but it has been speculated that Hcy may form illegitimate sulfide bonds with cysteine residues on secreted proteins, perturbing their folding and inducing ER stress.

In yeast, the UPR upregulates not only chaperones and components of the secretory apparatus but also enzymes involved in lipid biosynthesis. This response has been interpreted teleologically as an attempt by the organism to increase the total capacity of the ER, which has a significant lipid component (19). In yeast, this adaptation is effected by the IRE1 pathway, which also controls the UPR, but vertebrates have evolved specific sensors for the sufficiency of the lipid component of their ER membranes. Signaling in the vertebrate pathway is initiated by the processing of inactive membrane-bound transcription factors known as sterol regulatory element binding proteins (SREBPs) to their active forms, an event triggered by limitation of the availability of lipid (20). Werstuck et al. find that in addition to UPR target genes, Hcy also activates the expression of genes such as *IPP isomerase* and *HMG-CoA reductase* that are known to be downstream of the SREBPs (2). The *SREBP-1* gene itself is activated by HHcy and that activation is inhibited by overexpression of BiP, indicating that the UPR likely contributes to this process. They suggest that the hepatic steatosis observed in HHcy mice (and severely affected HHcy humans) may be due to increased signaling in the SREBP pathway (2). Their findings suggest that in mammalian cells too the UPR can upregulate lipid biosynthesis by increasing the level of SREBP mRNA and protein. And such upregulation of SREBP signaling promotes the import of lipoproteins and synthesis of cholesterol and lipids. While these observations readily explain the hepatic accumulation of lipid, their significance to vascular disease in HHcy remains to be explored. In particular, it would be interesting to determine whether Hcy-mediated ER stress signaling impacts on the uptake of lipoproteins by endothelial cells.

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