Homocysteine: to measure or not to measure? Focus on “Functional NMDA receptors in rat erythrocytes”

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The study by Makrho and colleagues, published in this issue (11), shows presence of N-methyl-D-aspartate (NMDA) receptors in different cell types and tissues from the rat: UT-7/Epo cells, bone marrow cells, and cerebellar tissue. Interestingly, peripheral blood also expressed NMDA receptors, which are more abundant in erythroid precursor cells and reticulocytes. These findings were validated by applying different methodologies, such as determination of the number of receptors by radiolabeling, Western blotting, immunohistochemistry, flow cytometry, ion transport with radiotracers, and several others, to demonstrate not only presence but also functionality of these receptors.

The study also addresses an important topic concerning the effect of homocysteine (HC) in cardiovascular disease (CVD). Numerous studies have been conducted in the past and are presently ongoing in this field due to the demonstrated harmful effects this amino acid has on several organ systems and, particularly, in the vascular system. Despite the attention paid to the subject by both the scientific community and funding agencies in this country and abroad, no clear evidence of a causal role of HC levels on certain clinical outcomes, especially regarding the general population, could be derived and the consensus is that more studies are needed to demonstrate this point. Epidemiological studies in the late 1990s reported that elevated plasma HC levels are a strong predictor of mortality in patients with coronary artery disease (CAD) (6, 13, 15) and are associated with higher risks of coronary, cerebral, and peripheral vascular disease (5, 14, 18). As a result of these investigations, treatment with folic acid, vitamin B12, and B6 was implemented, at different doses and combinations, to lower blood HC. Subsequently, large-scale randomized, placebo-controlled clinical trials were initiated to assess whether there was a causal relationship between elevated HC and CVD and stroke (1, 3, 5, 8, 9, 16, 19). However, fortification of flour for bread and cereal grain products with folic acid, since January 1998 (9), has rendered the HC hypothesis untenable due to the significant effect that folate fortification had on the statistical power of ongoing clinical trials (3, 4). Nonetheless, despite the negative results for the general population and for patients with ischemic stroke (10, 16), some high-risk patient groups (6, 7), such as healthy postmenopausal women (14), renal transplant recipients (3), patients with the relatively common genetic methyltetrahydrofolate reductase (MTHFR) mutation (18), patients with previous transient ischemic attack or stroke (8, 9), premature atherosclerosis disease (17), type 2 diabetes mellitus (15), peripheral vascular disease (see references in Ref. 7), and others do not seem to be protected by the average HC lowering therapy (1, 2, 5, 6, 8–10, 12, 17), and their HC plasma levels should be regularly monitored.

In addition to the effect of flour fortification with folate on clinical outcomes, other factors can affect the levels of HC in blood, such as genetic, physiological, and lifestyle factors, disease states, and drugs (8). Furthermore, results from clinical trials may be affected by the techniques and conditions of sample collection to measure plasma HC, the different methodologies used, and the interpretation of the evidence found in different types of studies such as observational, genetic, dietary, and large randomized controlled trials (8). Accordingly, lowering of HC by implementation of folic acid-based multivitamin therapy produced inconclusive results in the prevention of clinical vascular events and thus routine HC testing and treatment with this therapy could not be recommended until new information was available (8). However, as indicated above, evidence of an association between elevated plasma HC level and cardiovascular risk exists, especially that obtained in large-scale epidemiological studies, and a search for new approaches to resolve this conflict is needed. Thus several groups of investigators have concentrated in elucidating the mechanisms involving plasma HC levels, HC lowering therapy, vascular function, and atherogenesis (2) and references therein.

Several effects of elevated plasma total HC have been observed in vivo and in vitro, with the two majorly reported being on atherogenesis and thrombogenesis (2, 8). Both in human and in animal models, HC has been shown to increase oxidative stress by promoting the production of reactive oxygen species (ROS), such as superoxide anion (O2·−), and of reactive nitrogen species (RNS), such as peroxynitrite (ONOO−). These actions of HC generate a pro-oxidative state that leads to activation of inflammatory mediators involved in signaling pathways and promote expression of several pro-inflammatory genes (2). While ROS and RNS are mostly involved in vasoconstriction and inflammation, nitric oxide (NO), at low concentrations (in the nM range), plays a major role in vasodilation. In turn, HC, by promoting uncoupling of endothelial NO synthase leads to a decrease in the production of NO and to an increase in O2− (2). These and additional effects derived from the HC biosynthetic pathway offer plausible explanations at the mechanistic level to understand the role of HC on endothelial and vascular function and thrombogenesis (2).

In line with the above mechanisms and findings, Makrho and colleagues (11) are reporting that homocysteic acid (HCA), which like HC binds to the NMDA receptor, stimulates ouabain-resistant and clotrimazole-sensitive K influx with an IC50 of 21.1 ± 0.78 μM. Data from this and other studies referenced in the study, indicate that the IC50 for glutamate binding to the brain and erythrocyte NMDA receptor is similar (about 90 μM in rat), and that the IC50 of the brain receptor for HC and HCA is about 14 μM. The concentrations of HC in

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normal healthy subjects is <15 μM, depending on the diet and other factors, and can increase to >100 μM in subjects with severe homocysteinemia (2). This suggests that NMDA receptors in peripheral blood will be preferentially regulated by HC and HCA, making this finding of high relevance in the search for mechanisms attempting to explain HC harmful effects in CVD. Activation of the NMDA receptor by its agonists induces entry of Ca into the cell (Fig. 3 in Ref. 11). An increase in intracellular Ca causes activation of the ouabain-resistant and clotrimazol-sensitive pathway (Fig. 4A in Ref. 11). Because under physiological conditions the electrochemical gradient for K is outwardly directed, activation of this pathway leads to cell shrinkage (see Fig. 6A in Ref. 11) and increases the potential for clot formation. Thus the study by Makrho and colleagues (11) appears to provide new mechanisms to explain the atherogenic and thrombogenic actions of plasma HCA and HC. The present study could also lead to the discovery of new and more reliable markers that could help further evaluation of the role HC plays in CVD and associated pathologies, as well as in testing the HC hypothesis. Although the experimental model used by Makrho and colleagues (11) is the rat, the findings have the potential for application to human, and extensive reference is provided by the authors for both the model and human pathology to strengthen the relevance of their study. Furthermore, the authors indicate that studies in human tissues are in progress, and this may shed further light on the mechanisms involving HC in CVD.

REFERENCES