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Review

On the mechanism of homocysteine pathophysiology and pathogenesis: a unifying hypothesis

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Summary. Studies have shown that hyperhomocysteinemia is an important and independent risk factor for a variety of human cardiovascular diseases. In this paper, a unifying hypothesis is proposed which suggests that hyperhomocysteinemia may exert its pathogenic effects largely through metabolic accumulation of S-adenosyl-*L*-homocysteine, a strong noncompetitive inhibitor of the catechol-*O*-methyltransferase (COMT)-mediated methylation metabolism of various catechol substrates (such as catecholamines and catechol estrogens). In the case of endogenous catecholamines in peripheral tissues, inhibition of their methylation by *S*-adenosyl-*L*-homocysteine will result in elevation of blood or tissue levels of catecholamines, and consequently, over-stimulation of the cardiovascular system's functions. Moreover, because the vasculature is constantly exposed to high levels of endogenous catecholamines (due to high levels of circulating neurohormone epinephrine plus rich innervation with sympathetic nerve terminals), vascular endothelial cells would incur chronic cumulative damage caused by the large amounts of the oxidative products (catechol quinones/semiquinones and oxyradicals) generated from endogenous catecholamines. This mechanistic explanation for the vascular toxicity of hyperhomocysteinemia is supported by many experimental findings, and it also fully agrees with the known protective effects of folate, vitamins B_6 and B_{12} in hyperhomocysteinemic patients. In addition, based on the predictable effects of hyperhomocysteinemia on the methylation of catecholamines in the central nervous system as well as on the methylation of catechol estrogens in estrogen target organs, it is also suggested that hyperhomocysteinemia is an important risk factor for the development of neurodegerative disorders (Parkinson's and Alzheimer's diseases) and estrogen-induced hormonal cancers. More studies are warranted to test these intriguing ideas.

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Introduction

Following Dr. K.S. McCully's pioneering observation some 30 years ago (McCully, 1969), human epidemiological as well as laboratory animal studies have confirmed that hyperhomocysteinemia is an important and independent risk factor for a variety of cardiovascular diseases (McCully and Wilson, 1975; Wilcken and Wilcken, 1976; Harker et al., 1982; Boers et al., 1985; Malinow et al, 1990; Clarke et al., 1991; Stampfer et al., 1992; Arnesen et al, 1995; Lentz et al., 1996; McCully, 1996). A number of plausible mechanisms have been proposed to explain the atherogenic actions of homocystenine, which include vascular endothelial dysfunction (van den Berg et al., 1995; Lentz et al, 1996; Tawakol et al., 1997), direct cytototoxic effects to vascular endothelial cells (Starkebaum and Harlan, 1986), diminished release of nitric oxide (Upchurch et al., 1997) and increased production of reactive oxygen species in vascular enthothelial cells (Startebaum et al., 1986), stimulation of the low-density lipoprotein oxidation (Hirano et al., 1994; Blom et al., 1995), promotion of platelet activation and enhanced coagulability (Harker et al., 1976), and increased proliferation of vascular smooth muscle cells (Tsai et al., 1994). However, the exact mechanism by which hyperhomocysteinemia causes atherogenesis as well as others cardiovascular diseases is still not clear. The lack of complete mechanistic understanding of homocysteine pathophysiology has hampered the development of effective prevention and treatment approaches. Here I propose a unifying hypothesis for the mechanism of homocysteine pathophysiology and pathogenesis on the basis of the following known facts: (i) homocysteine is an immediate

Abbreviations: COMT, catechol-*O*-methyltransferase; SAM, *S*-adenosyl-*L*-methionine; SAH, *S*-adenosyl-*L*-homocysteine; B_6, vitamin B_6; B_{12}, vitamin B_{12}

precursor for the biosynthesis of *S*-adenosyl-*L*-homocysteine (SAH) (Ueland, 1982); (ii) SAH is a strong, noncompetitive inhibitor of the COMT-mediated *O*-methylation metabolism of various catechol substrates (Ueland, 1982; Zhu and Liehr, 1996; Zhu et al., 2000); and (iii) the oxidation (redox cycling) of endogenous catechols generates large amounts of chemically-reactive products (quinone/semiquinone intermediates and oxyradicals) that are highly toxic to the surrounding cells (Bolton et al., 2000). This hypothesis predicts that hyperhomocysteinemia is not only an important risk factor for the development of cardiovascular diseases, but it is also an important risk factor for the development of neurodegerative disorders (e.g., Parkinson's and Alzheimer's diseases) as well as estrogen-related hormonal cancers.

COMT-mediated *O*-methylation of catecholamines and its regulation by SAH

Although COMT is almost ubiquitously present in the body, its distribution in various tissues or cells largely corresponds to their level of exposure to various catechol substrates. Notably, the vasculature (particularly vascular endothelial cells) is constantly exposed to very high levels of endogenous catecholamines because of exposure to the circulating neurohormone epinephrine and rich innervation with sympathetic nerve terminals. As expected, COMT is abundantly present in cardiovascular tissues and erythrocytes. Similarly, high levels of the COMT activity are also present in certain regions of the brain where dopamine or norepinephrine is used as neurotransmitter (Kastner et al., 1994). There is evidence showing that in the human brain, large amounts of COMT are densely contained in the cell body of certain catecholamine-containing neurons as well as in the neighboring glial cells (Kastner et al., 1994).

It is well known that COMT catalyzes the metabolic *O*-methylation of organic chemicals with a free catechol structure. Generally, COMT has very low substrate specificity, i.e., it catalyzes the metabolic *O*-methylation of a wide variety of substrates, such as endogenous catecholamines (neurotransmitters/neurohormone) and catechol estogens, as well as various catechol-containing xenobiotics that are ingested into the body (Zhu et al., 1994, 2000, 2001; Zhu and Liehr, 1996). The COMT-mediated methylation metabolism of endogenous catecholamines and catechol estrogens not only inactivates their neurotransmitter and hormone activities, but it is also largely responsible for eliminating the potential chemical reactivity and cytotoxicity of these catechols. Understandably, the COMT-mediated metabolism of these endogenous bioactive catechols is better not easily disturbed by the presence of various catechol-containing xenobiotics (they are co-substrates and usually are competitive inhibitors of the enzyme). Otherwise, the physiological functions of endogenous bioactive catechols may often fluctuate wildly and undesirably. As explained in the *Appendix*, owing to the

APPENDIX

Kinetic properties associated with the low-affinity, high-capacity COMT

The soluble and membrane-bound COMT (S-COMT and MB-COMT, respectively) in different tissues generally have high K_M (Michaelis-Menten constant) and also high V_{MAX} (maximal velocity) for the methylation metabolism of various catechol substrates. Comparing S-COMT and MB-COMT, the former usually has a higher K_M (lower affinity) than the latter. Nevertheless, the K_M values for both isoforms are usually up to orders of magnitude higher than the available in vivo concentrations of most substrates. For instance, the K_M values of hepatic S-COMT are ~1 mM for catecholamines and ~20 μM for catechol estrogens (Zhu and Liehr, 1993; Zhu et al., 1994) when tested in vitro under optimized reaction conditions, whereas the in vivo available concentrations of these two classes of endogenous catechols are much lower than 1 μM and 1 nM (Kono et al., 1982; Ludwig et al., 1988), respectively, which are at least 3-orders of magnitude lower than their respective K_M values.

Because most catechol substrates have in-vivo concentrations orders of magnitude lower than their corresponding K_M values for COMT, the rate of their *O*-methylation metabolism when they are co-present at these low concentrations will almost be the same as the rate of metabolism when each individual substrate is present alone. Fig. 1 depicts a hypothetical situation where 4 substrates (S_1, S_2, S_3, and S_4) for the same enzyme are co-present. According to the equation listed, if the concentrations of these 4 substrate are 1/1000 or 1/100 of their corresponding K_M values, then the rate of their metabolism will be 99.7% or 97.1%, respectively, of the control metabolic rate when each substrate is present alone. However, if the same absolute concentrations of these 4 substrates are present but the enzyme has a 1000-times lower K_M value for each substrate, then the rate of their metabolism will only be 40.0% or 26.8%, respectively, of the control metabolic rate when each substrate is present alone. Therefore, the presence of multiple substrates at the same concentrations will exert a much stronger competitive inhibition of the metabolism of each substrate if the metabolizing enzyme has a much lower K_M value.

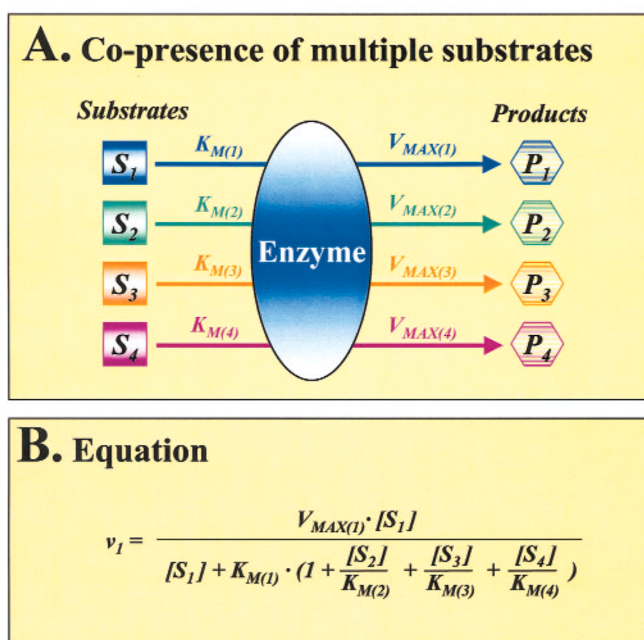
However, for a high-K_M metabolizing isozyme, the rate of metabolism of a substrate at its low concentrations ($\ll K_M$) would be much slower than the rate of metabolism with a low-K_M isozyme if the two isozymes are present in equimolar quantities. The slower rate of metabolism with the high-K_M isozyme could be fully compensated for by increasing the amount of the enzyme protein present in a given cell or tissue (i.e., by increasing the V_{MAX}). In fact, this is probably the main reason why most low-specificity metabolizing enzymes (such as hepatic cytochrome p450 enzymes and glucuronosyltransferases) often have high capacity (high V_{MAX}) besides having low affinity (high K_M) for their multiple substrates.

Therefore, owing to the high-K_M and high-V_{MAX} kinetic properties of COMT, the methylation metabolism of each catechol substrate can almost remain undisturbed even when multiple substrates of the enzyme are co-present. Note that the unique physiological advantages of the low-affinity, high-capacity metabolizing enzymes have not been adequately appreciated in the past and were frequently misunderstood or misconstrued in scientific publications.

In comparison, the situation would be entirely different when a noncompetitive inhibitor (or multiple noncompetitive inhibitors) of a metabolizing enzyme is present. Fig. 2 depicts the inhibition of enzyme-mediated metabolism by a noncompetitive inhibitor. According to the

APPENDIX (continuation)

equation used for calculating the relative fraction (%) of substrate metabolism remained when a noncompetitive inhibitor is present, it is clear that a noncompetitive inhibitor at a given concentration will provide exactly the same degree (percentage) of inhibition regardless of the substrate used, its concentration present, and its K_M value. Stated differently, increasing the concentrations of a noncompetitive inhibitor of COMT would inhibit the rate of metabolism of all substrates to the same degrees. The only relevant parameters here are the K_I of the noncompetitive inhibitor and its available concentrations. These two parameters (more precisely, the ratio of the two: $[I]/K_I$) will determine the degree of enzyme inhibition. Note that the same kinetic characteristics of enzyme inhibition will be seen when multiple noncompetitive inhibitors are co-present.



$$v_1 = \frac{V_{MAX(1)} \cdot [S_1]}{[S_1] + K_{M(1)} \cdot \left(1 + \frac{[S_2]}{K_{M(2)}} + \frac{[S_3]}{K_{M(3)}} + \frac{[S_4]}{K_{M(4)}}\right)}$$

Fig. 1. A hypothetical situation where 4 substrates (S_1, S_2, S_3, and S_3) for the enzyme are co-present (panel A). The corresponding K_M and V_{MAX} values for the metabolism of each of the substrates when present alone are: $K_{M(1)}$ and $V^{MAX}{}_{(1)}$ for S_1; $K_{M(2)}$ and $V_{MAX(2)}$ for S_2; $K_{M(3)}$ and $V_{MAX(3)}$ for S_3; and $K_{M(4)}$ and $V_{MAX(4)}$ for S_4. When these 4 substrates are co-present, they are also competitive inhibitors of each other's metabolism by the same enzyme. The equation for calculating the velocity (v) of the metabolism of any one substrate (such as S_1) when other 3 substrates are also co-present is shown in **panel B**.

It is of interest to note that SAH is an endogenous noncompetitive inhibitor of COMT, and its available intracellular concentrations are usually near or even higher than its K_I value, thus making it a crucial endogenous modulator of COMT-mediated *O*-methylation metabolism of endogenous and exogenous catechols.

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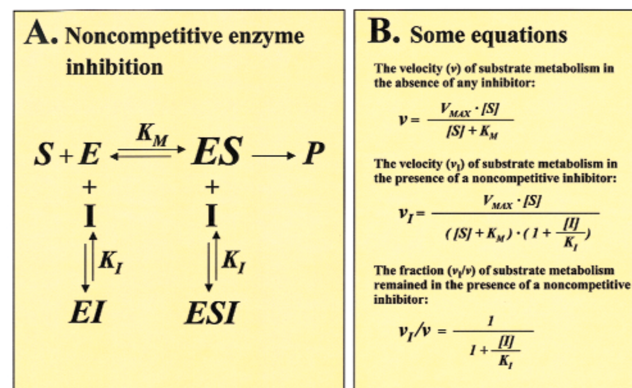

$$v = \frac{V_{MAX} \cdot [S]}{[S] + K_M}$$

$$v_I = \frac{V_{MAX} \cdot [S]}{([S] + K_M) \cdot (1 + \frac{[I]}{K_I})}$$

$$v_I/v = \frac{1}{1 + \frac{[I]}{K_I}}$$

Fig. 2. Schematic illustration of the interaction of an enzyme with the substrate S and the noncompetitive inhibitor I **(panel A)**. **Panel B** lists the equations for calculating the metabolic velocity in the absence or presence of a noncompetitive inhibitor as well as for calculating the relative fraction (%) of substrate metabolism remained when a noncompetitive inhibitor is present.

low affinity (high K_M) and high-capacity (high V_{MAX}) of the COMT for various catechol substrates, their metabolic *O*-methylation can almost remain undisturbed even when multiple substrates are co-present at physiologically-relevant low concentrations.

During the COMT-mediated *O*-methylation of catecholamines or other catechol substrates, SAM (the methyl donor) is converted to SAH after donating the methyl group to the substrate. It has been known for many years that SAH is an endogenous inhibitor of the COMT-mediated *O*-methylation of endogenous as well as exogenous catechols (Ueland, 1982; Zhu and Liehr, 1994; Zhu et al., 2000). Earlier studies indicated that the K_I value of SAH for COMT was lower than the K_M value of SAM for the enzyme (Hoffman et al., 1980). Using human placental cytosolic COMT, Zhu et al. (2000) recently showed that the apparent K_M value for SAM was ~24 μM, whereas the apparent K_I value for

SAH was ~4 μM (ranging from 3.2-5.2 μM), suggesting that the human placental COMT has a significantly higher affinity for SAH than for SAM. Notably, the rodent COMT also has a higher apparent K_I value for SAH (Zhu et al., 1994, 2001; Zhu and Liehr, 1996). As indicated in several earlier studies (Manteuffel-Cymborowska et al., 1992; Eloranta, 1997; Caudill et al., 2001), the concentrations of SAH in several rodent tissues could vary markedly, ranging from 2.5-30 nmol/g wet tissue under different conditions.

Mechanistically, Zhu and Liehr (1996) earlier demonstrated that SAH could competitively inhibit the ability of SAM to function as a methyl donor during the COMT-mediated methylation metabolism of 2-hydroxyestradiol, a representative endogenous catechol substrate (Fig. 1). This data suggested that SAM and SAH interact with the same binding site (or binding pocket) on the COMT protein, which is fully consistent with the X-ray crystallography data (Vidgren et al., 1994) and the data from computational modeling studies (Vidgre, 1998). Our further kinetic analysis demonstrated that SAH was a pure noncompetitive inhibitor with respect to the COMT-mediated formation of methylated products (Fig. 2). The mechanistic explanation for the observed enzyme kinetics is schematically depicted in Fig. 3.

As explained in the *Appendix*, a noncompetitive inhibitor at a given concentration will provide the same degree (or percentage) of inhibition of an enzyme regardless of the substrate used, its concentrations present, and its K_M value. Because SAH is a strong noncompetitive inhibitior of COMT, increasing the cellular concentrations of SAH would suppress the COMT-mediated methylation metabolism of endogenous and exogenous catechols in a concentration-dependent manner, and subsequently, increase their blood and tissue levels. Taking the peripheral catecholamines as an example, sustained elevation of these bioactive catechols is expected to bring about a series of pathogenic cardiovascular changes largely through the following two general mechanisms:

First, norepinephrine (a neurotransmitter) and epinephrine (a neurohormone) are known powerful stimulators of the cardiovascular system. Mechanistically, they exert their actions through activation of the postsynaptic ß$_1$-adrenoceptors in the heart to increase its chronotropic and inotropic actions, and through activation of the postsynaptic α-adrenoceptors in the vascular smooth muscle cells to causes strong vasoconstriction. In addition, activation of renal ß$_1$-adrenoceptors in the juxtaglumerolar cells stimulates renin release and subsequently activates the renin-angiotensin-aldosterone system, resulting in increased vasoconstriction and volume overload. Even if

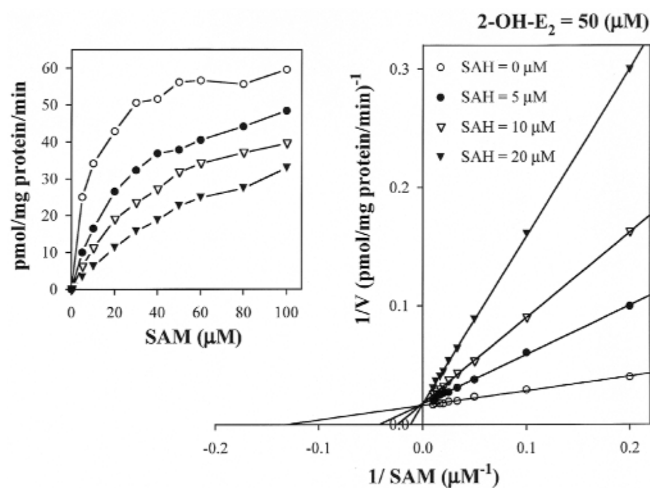

Fig. 1. Enzyme kinetic data showing that SAH competitively inhibits SAM as a methyl donor for the COMT-mediated *O*-methylation reaction (Zhu and Liehr, 1996). The left-upper panel shows the rate of COMT-mediated methylation of 50 μM 2-hydroxyestradiol (2-OH-E_2) as a function of increasing SAM concentrations in the absence or presence of SAH. The right panel shows the double-reciprocal plot for the data. Adopted from Zhu and Liehr (1996).

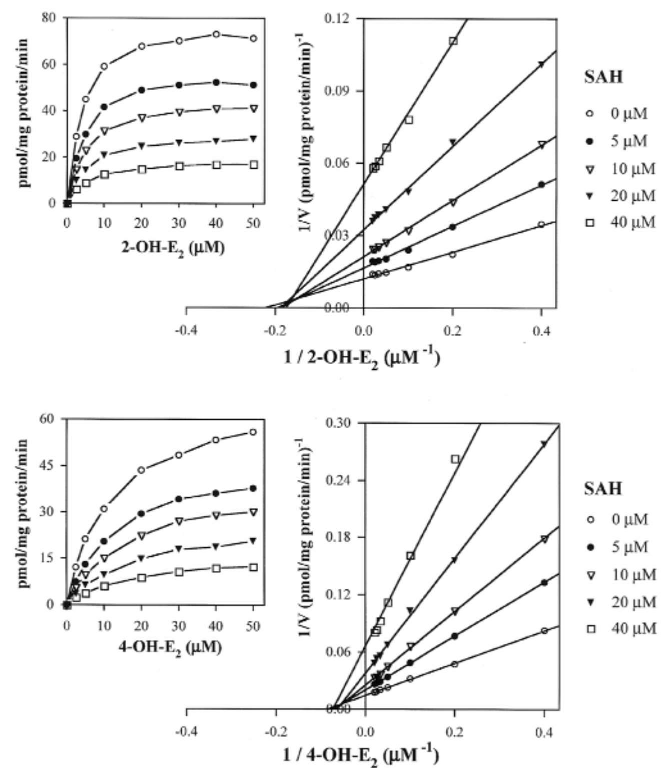

Fig. 2. Enzyme kinetic data showing that SAH is a noncompetitive inhibitor for the formation of methylated products (Zhu and Liehr, 1996). The left-upper panel shows the rate of COMT-mediated methylation of increasing concentrations (from 2.5 to 50 μM) of 2-hydroxyestradiol (2-OH-E_2) and 4-hydroxyestradiol (4-OH-E_2) in the absence or presence of different concentrations of SAH. Note that a fixed concentration (50 μM) of SAM was used as the methyl donor when different substrate concentrations were assayed. The right panel shows the double-reciprocal plot of the data. Adopted from Zhu and Liehr (1996).

we assume that the adrenoceptor-mediated activation of the cardiovascular and renin-angiotensin-aldosterone systems by elevated levels of endogenous catecholamines may be partially desensitized over time (due to down-regulation of the postsynaptic $ß_1$- and α-adrenoceptors), sustained overstimulation of these systems has long been recognized as a crucial risk factor for a variety of cardiovascular diseases, such as hypertension, coronary heart disease, and congestive heart failure. Numerous studies have shown that clinical use of $ß_1$- and/or α_1-adrenoceptor antagonists is highly effective in alleviating these medical conditions and also in curbing their progression in humans.

Second, the endogenous catecholamines are potentially reactive molecules. Elevated tissue levels of catecholamines will result in increased formation of chemically reactive catechol quinones/semiquinones and oxyradicals (hydroxy radicals and superoxide radicals) (Bolton et al., 2000). One of the metabolic pathways responsible for the formation of reactive intermediates is the redox cycling between catecholamines (or their catechol-containing metabolites) and their quinone/semiquinone intermediates (Stokes et al., 1999; Bolton et al., 2000). A variety of oxidizing enzymes (e.g., cytochrome P450 enzymes, tyrosine hydroxylase, tyrosinase, and lactoperoxidase) can serve as catalysts for the redox cycling reactions (Bolton et al., 2000). Moreover, these oxidation reactions can also occur automatically in the absence of enzymes (a process called "autooxidation"). Many earlier studies have demonstrated that the reactive catecholamine quinones/semiquinones and oxyradicals are extremely toxic to the cells (Stokes et al., 1999; Bolton et al., 2000).

In addition, hydrogen peroxide can be generated during the monoamine oxidase (MAO)-mediated metabolism of catecholamines. Hydrogen peroxide, in the presence of ferrous ion, may generate hydroxyl free radicals via the Fenton reaction (Cohen, 2000). The multiple oxidative metabolic pathways leading to the generation of a variety of free radicals and reactive catecholamine intermediates are depicted in Fig. 4.

In summary, SAH at elevated concentrations is a strong noncompetitive inhibitor of the COMT-mediated *O*-methylation metabolism of endogenous and exogenous catechols. Decreased *O*-methylation metabolism of the catechol substrates would increase their blood and tissue concentrations, and subsequently would enhance their biological functions as well as cytotoxicity. Depending on the types of cells affected and different catechols (catecholamines vs. catechol estrogens) involved, such oxidative damage could be important etiological factors for the development of

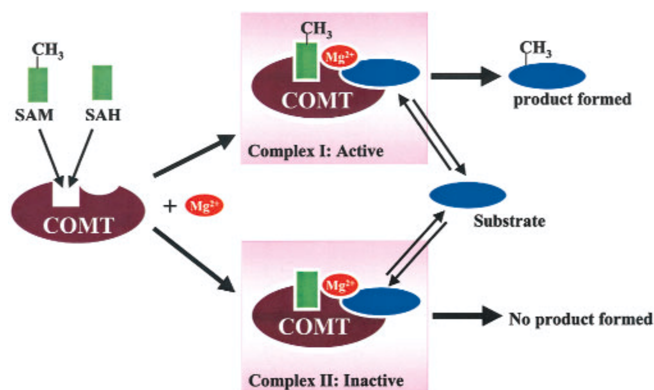

Fig. 3. Schematic illustration explaining why SAH is a noncompetitive inhibitor of COMT-mediated methylation reaction. As shown, COMT is a single protein enzyme, and it has similar binding affinities for both SAM (the methyl donor) and SAH (the demethylated product of SAM). When the enzyme is bound with SAM (complex 1), the enzyme is in the active state, capable of methylating the substrate. However, when the enzyme is associated with SAH (complex 2), it becomes inactive and cannot complete the methylation reaction because SAH cannot provide the methyl group. Hence, when the concentration of SAH increases, SAH will competitively inhibit SAM from binding to COMT and will shift more enzymes to the inactive state (complex 2), consequently resulting in a decrease in the V_{max} but no change in the K_M since the active enzymes (complex 1) remaining will still have the same affinity for the substrate. A decrease in the V_{max} but no change in the K_M is typical of a "noncompetitive" enzyme inhibition.

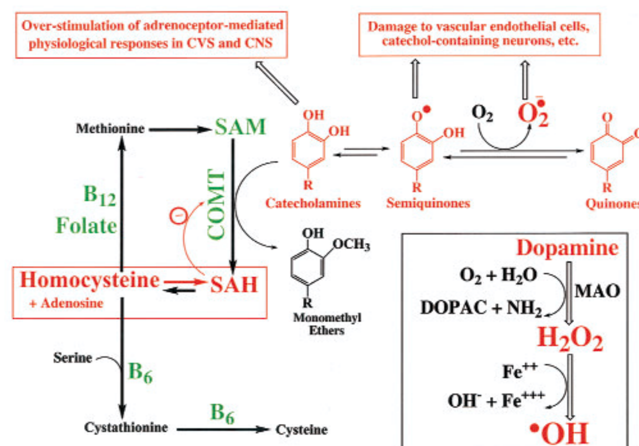

Fig. 4. Proposed mechanism of homocysteine pathophysiology and pathogenesis based on accumulation of intracellular SAH. SAH can strongly and noncompetitively inhibit the COMT-mediated *O*-methylation of bioactive catechols (such as catecholamines and catechol estrogens). In the case of catecholamines, inhibition of their methylation by SAH would result in their accumulation, and subsequently over-stimulation of the adrenoceptor-mediated functions of the cardiovascular system (CVS) and/or the central nervous system (CNS). In addition, elevated levels of catecholamines would also lead to strong cytotoxicity due to formation of catechol quinone/semiquinone intermediates and oxyradicals. As shown, folate, vitamins B_6 and B_{12} are key cofactors for the metabolic pathways involved in the regulation of the cellular levels of homocysteine and SAH. The presence of adequate amounts of cellular folate, vitamins B_6 and B_{12} would be greatly beneficial for reducing homocysteine levels through various catabolic pathways, and ultimately, reducing the intracellular levels of SAH. In addition, catecholamines may also lead to metabolic formation of hydroxy radicals via the monoamine oxidase (MAO)-mediated pathway. The lower-right inset depicts the MAO-mediated formation of free radicals from dopamine. DOPAC is the abbreviation for 3,4-dihyroxyphenylacetic acid, an endogenous dopamine metabolite.

cardiovascular diseases, neurodegenerative disorders, as well as estrogen-induced hormonal cancers (more discussion is provided below).

Altered SAH biosynthesis and catabolism is a key event underlying homocysteine pathophysiology and pathogenesis

Inside the cells, SAH is recycled to form SAM via enzymatic conversion first to homocysteine and then to methionine as intermediates (Fig. 4). The conversion of SAH to homocysteine is catalyzed by SAH hydrolase, and the further conversion of homocysteine to methionine is mainly catalyzed by 5-homocysteine methyltransferase using vitamin B_{12} and folate as cofactors (Durand et al., 2001). Homocysteine can also react with serine to form cystathionine and further to cysteine, reactions that are vitamin B_6-dependent (Durand et al., 2001).

Although SAH hydrolase catalyzes the reversible conversion between SAH and homocysteine, the metabolic flow under normal physiological conditions proceeds in the hydrolytic direction because homocysteine or adenosine or both is/are usually rapidly metabolized in the cells (de la Haba and Cantoni, 1959). However, when the concentrations of intracellular homocysteine and/or adenosine are accumulated under certain conditions, the reaction will favor SAH biosynthesis. An earlier study (de la Haba and Cantoni, 1959) reported that the equilibrium constant for this reaction is ~1 μM, which means,

$$\text{Equilibrium constant} = [\text{Adosine}]\,[\text{Homocysteine}] / [\text{SAH}] = 1\ \mu\text{M}.$$

This mathematical relationship indicates that the enzymatic catalysis will be directed toward SAH hydrolysis when the concentrations of either homocysteine or adenosine or both are low. However, when homocysteine (or adenosine) accumulates in the cell or after increased intake of exogenous homocysteine, the reaction catalyzed by SAH hydrolase will be directed toward SAH biosynthesis, and the enzymatic degradation of SAH will be reduced. Consequently, high cellular levels of SAH will be resulted. This has been demonstrated in cultured or isolated cells (Kredich and Martin, 1977; Johnson and Kredich, 1979; Kredich and Hershfield, 1979), in perfused liver or heart (Hoffman et al., 1980), and in whole animals (Schatz et al., 1981). As discussed earlier, elevated levels of SAH resulting from hyperhomocysteinemia would noncompetitively and strongly inhibit the COMT-mediated methylation metabolism of endogenous catecholamines (as well as other catechol substrates), subsequently resulting in over-stimulation of the functions of the cardiovascular system and even causing oxidative damage to the surrounding cells that are exposed to elevated levels of endogenous catecholamines.

As noted earlier, the vascular endothelial cells are exposed to high levels of endogenous catecholamines because the vasculature is richly innervated with peripheral sympathetic nerves and is constantly exposed to the circulating neurohormone epinephrine. Moreover, the endothelial cells are also constantly exposed to high concentrations of exogenous catechols that are ingested into the body and present in circulation. Similarly, there are many types of neuronal cells in the central nervous system that either contain or are constantly exposed to large amounts of the catecholamine neurotransmitters. It is expected that these as well as other cells in the body that are constantly exposed to high levels of endogenous catecholamines would be among the first-line targets that may manifest signs of cytotoxicity when the COMT metabolic pathway is noncompetitively and strongly inhibited by SAH and when increased redox cycling of endogenous catecholamines is resulted. Marked increase in catecholamine-mediated oxidative damage to vascular endothelial cells may contribute to the development of occlusive vascular disorders in humans. Likewise, sustained increase in oxidative damage to neuronal cells in certain parts of the brain rich in catecholamine neurotransmitters and/or low in COMT activity may contribute to the development of neurodegenerative disorders such as Parkinson's disease (in particular) and Alzheimer's disease. In this context, it is noteworthy that an earlier immunohistochemical study (Kastner et al., 1994) indicated that the COMT activity likely is indigenously very low in the dopaminergic neurons of human substantia nigra pars compacta. This might be an important intrinsic risk factor that determines the susceptibility to Parkinson's disease.

There are several lines of evidence that supports the proposed mechanistic explanation of homocysteine pathophysiology and pathogenesis. First, studies have indicated that the vascular lesions caused by hyperhomocysteinemia are, in many ways, characteristic of free radical-mediated damage (Starkebaum and Harlan, 1986; Clarke et al., 1992). The observation that chronic administration of antioxidants such as vitamin E partially protected against homocysteine-induced vascular damage (Raghuveer et al., 2001) provides support for this suggestion. Second, because the two major clinical consequences that most frequently accompany hereditary hyperhomocysteinemia are mental retardation and severe atherosclerosis (McCully, 1969; McCully and Wilson, 1975; Wilcken and Wilcken, 1976), they are among the predicted first-line targets for homocysteine-mediated cytotoxic damage. Third, a recent study reported that elevated blood levels of SAH are more sensitive as an indicator for human cardiovascular risk than homocysteine (Kerins et al., 2001). This finding fully agrees with the proposed mechanism for homocysteine pathophysiology and pathogenesis. In addition, it is of interest to also note that an earlier study showed that treatment of mice with a high dose of testosterone strongly increased the hepatic levels of SAH (Manteuffel-Cymborowska, 1992). This

observation, along with the proposed hypothesis, offers insights into the pathogenic mechanism underlying the well-known cardiac toxicity associated with chronic overdose of anabolic steroids in human subjects.

Another line of supporting evidence is the perfect agreement between the proposed mechanistic explanation and the well-known effects of folate, vitamins B_6 and B_{12} in their protection against the pathogenic changes associated with hyperhomocysteinemia (Verhoef et al., 1996; Clarke and Armitage, 2000; Varela-Moreiras, 2001). Vitamin B_{12} and folate are key cofactors for the enzymatic conversion of homocysteine to methionine and further to SAM (see Fig. 4). When their cellular supply is inadequate, the enzymatic conversion of homocysteine to methionine will be reduced, leading to accumulation of homocysteine and decreased biosynthesis of SAM. Similarly, decreased conversion of homocysteine to cysteine (a vitamin B_6-dependent pathway) can also lead to accumulation of homocysteine. As discussed earlier, elevated levels of homocysteine inhibit of the enzymatic conversion of SAH to homocysteine and consequently result in SAH accumulation. Elevated concentrations of SAH or decreased availability of SAM or both would hamper the COMT-mediated *O*-methylation of catechol substrates. On the other hand, supplementing exogenous vitamin B_{12} and/or folate would facilitate the conversion of homocysteine to methionine, and consequently they would decrease the levels of SAH and also increase the formation of SAM. Similarly, supplementing vitamin B_6 would accelerate the conversion of homocysteine to cystathionine and cysteine, a diverging pathway that would help reduce homocysteine and SAH accumulation, thereby alleviating the problem. These explanations are in accord with the findings from epidemiological studies (Verhoef et al., 1996; Clarke and Armitage, 2000; Varela-Moreiras, 2001) showing that the plasma homocysteine levels correlated negatively with the plasma levels of folate, vitamins B_6 and B_{12}, and also with the dietary folate and vitamin B_6 intake.

Concluding remarks

The earlier discovery of hypercholesterolemia as an important risk factor for human cardiovascular diseases has led to tremendous improvements in the treatment and prevention of these medical conditions. Understandably, enormous scientific interest has also been developed toward the finding that hyperhomocysteinemia is another important and independent risk factor for human cardiovascular diseases because it is expected to open up new avenues for their treatment and prevention.

I proposed a unifying hypothesis that homocysteine may exert its pathogenic effects largely through metabolic accumulation of SAH, a strong noncompetitive inhibitor of the COMT-mediated methylation metabolism of endogenous and exogenous catechols. This hypothesis provides a sound mechanistic explanation for the cytotoxicity associated with hyperhomocysteinemia and its contributing role in the pathogenesis of cardiovascular and neurodegenerative disorders. This mechanistic explanation is consistent with and is also strongly supported by most experimental observations. Importantly, this hypothesis provides a perfect explanation for the known effects of folate, vitamins B_6 and B_{12} in their strong protection against hyperhomocysteinemia-associated pathogenic changes.

It should also be noted that elevated levels of intracellular homocysteine (and eventually elevated levels of intracellular SAH) will not only inhibit the COMT-mediated *O*-methylation of catecholamines, but they will also inhibit the *O*-methylation of catechol estrogens (e.g., 2-hydroxyestradiol and 4-hydroxyestradiol). Such an inhibition will result in decreased formation of 2-methoxyestradiol, a strong antiangiogenic and anticancer agent (Zhu and Conney, 1998), and increased accumulation of the procarcinogenic 4-hydroxyestradiol (Liehr, 2000). Both of these effects are expected to contribute importantly to the development of estrogen-induced hormonal cancers. Notably, increased incidence of hormonal cancer has not been reported in hereditary hyperhomocysteinemia, which, in a large part, might have been due to the fact that these patients usually die at a very early age before the cancer is fully developed. It will be of considerable interest to determine whether chronic elevation of blood levels of homocysteine indeed constitutes a significant risk factor for human hormonal cancers.

Lastly, it is of note that although there is considerable experimental evidence for the pathogenic role of hyperhomocysteninemia, almost nothing is known at present about the potential pathogenic role of elevated intracellular levels of adenosine, which, according to the proposed hypothesis, may share a similar mechanism of action to that of the intracellular homocysteine. More research is warranted to test these intriguing hypotheses. Studies in this area may enhance our understanding of the pathogenic mechanism(s) for homocysteine, and possibly also for adenosine, and ultimately, may lead to improved treatment and prevention of the intracellular homocysteine/adenosine-mediated pathogenic changes.

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