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The Adenosine Receptor Agonist 5'-*N*-Ethylcarboxamide-Adenosine Increases Mouse Serum Total Homocysteine Levels, Which Is a Risk Factor for Cardiovascular Diseases

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Abstract

An increase in total homocysteine (Hcy) levels (protein-bound and free Hcy in the serum) has been identified as a risk factor for vascular diseases. Hcy is a product of the methionine cycle and is a precursor of glutathione in the transsulfuration pathway. The methionine cycle mainly occurs in the liver, with Hcy being exported out of the liver and subsequently bound to serum proteins. When the non-specific adenosine receptor agonist 5'-*N*-ethylcarboxamide-adenosine (NECA; 0.1 or 0.3 mg/kg body weight) was intraperitoneally administered to mice that had been fasted for 16 h, total Hcy levels in the serum significantly increased 1 h after its administration. The NECA treatment may have inhibited transsulfuration because glutathione levels were significantly decreased in the liver. After the intraperitoneal administration of a high dose of NECA (0.3 mg/kg body weight), elevations in total Hcy levels in the serum continued for up to 10 h. The mRNA expression of methionine metabolic enzymes in the liver was significantly reduced 6 h after the administration of NECA. NECA-induced elevations in total serum Hcy levels may be maintained in the long term through the attenuated expression of methionine metabolic enzymes.

Keywords

Adenosine, 5'-*N*-Ethylcarboxamide-Adenosine, Glutathione, Homocysteine

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1. Introduction

An increase in total serum homocysteine levels (total Hcy: serum protein-bound and free Hcy) has been identified as a risk factor for cardiovascular disease [1] [2] and liver fibrosis [3]. The normal range of total Hcy in adults is typically 5 - 15 μM, with the mean level being approximately 10 μM [2]. Plasma Hcy concentrations were previously found to be strongly associated with the presence and number of small infarctions, or infarction of the putamen in elderly diabetic patients [4]. High levels of Hcy have been shown to induce endoplasmic reticulum (ER) stress and increase the production of reactive oxygen species (ROS) [5]. Hcy has strong reducibility and modifies disulfide bonds in proteins. Only 1% to 2% of Hcy occurs as thiol homocysteine in the serum; 75% of Hcy has been suggested to bind to proteins through disulfide bonds with protein cysteines [6]. Hcy is formed as an intermediary in methionine metabolism [7] [8]. Methionine metabolism mainly occurs in the livers of mammals. Methionine receives an adenosine group from ATP to become *S*-adenosylmethionine (AdoMet) in the methionine cycle. This reaction is catalyzed in the liver by liver-specific methionine adenosyltransferase I/III (MAT I/III), which is encoded by the methionine adenosyltransferase 1A (*MAT1A)* gene [9]. AdoMet then transfers its methyl group to a large number of compounds, a process that is catalyzed by various methyltransferases (e.g., glycine *N*-methyltransferase: GNMT; DNA methyltransferase; phosphatidylethanolamine *N*-methyltransferase), to produce *S*-adenosylhomocysteine (AdoHcy). Hcy is formed from AdoHcy by AdoHcy hydrolase (SAHH). The reaction that generates Hcy from AdoHcy is reversible, and AdoHcy from Hcy is shown to be thermodynamically favored over the synthesis of Hcy [10]. A previous study reported that Hcy levels were very low in the liver [11]. This reaction then proceeds toward the synthesis of Hcy when the products (Hcy and adenosine) are removed by further metabolism [12]. Three enzymes metabolize Hcy, with the betaine-homocysteine *S*-methyltransferase (BHMT) and methionine synthase (MS) reactions both yielding methionine. A large proportion of Hcy in the liver is remethylated by BHMT [3]. The third enzyme, cystathionine *β*-synthase (CBS) catalyzes Hcy to cystathionine in the transsulfuration pathway. Previous studies of whole body methionine kinetics demonstrated that 62% of Hcy was converted to cystathionine during each cycle in males fed a basal diet, resulting in the production of glutathione (GSH), while 38% of Hcy was remethylated to methionine [13]. Hcy is located at an important regulatory branch point: remethylation to methionine; conversion to cystathionine; export from the cells.

A decrease in intracellular ATP levels, accompanied by the accumulation of 5'-AMP and subsequently adenosine, is known to follow ischemia. Adenosine levels in interstitial fluids were shown to increase 100 - 1000-fold from basal levels (10 - 300 nM) with ischemia [14]. Furthermore, adenosine levels in hepatocytes were increased by a hypoxic challenge, with excess amounts of adenosine being exported out of cells [14]. Adenosine levels were also found to increase 10-fold due to hypoxia, stress, and inflammation [15]. Adenosine has been shown to activate A1, A2a, and A3 receptors with EC_{50} values in the range of 0.2 - 0.7 μM, and also A2b receptors with an EC_{50} of 24 μM [16]. A1 and A3 receptors have been classified as adenylate cyclase inhibitory receptors, and A2a and A2b receptors as adenylate cyclase-activating receptors [17]. The activation of adenosine receptors accompanied by ischemia may increase total Hcy levels in the serum because hepatic ischemia is known to decrease the content of GSH and activity of MAT [18].

We previously reported that the non-specific adenosine receptor agonist 5'-*N*-ethylcarboxamide-adenosine (NECA) increased serum glucose levels and the expression of a glucogenic enzyme (glucose 6-phosphatase) in the liver [19] [20]. Based on the dose of NECA administered in these studies and plasma concentrations after the administration of other adenosine agonists [21], it was inferred that the serum NECA concentration was in the μM range and also that NECA activated adenosine A2b receptors. In the present study, we measured methionine metabolites, including Hcy, in NECA-treated mice in order to determine whether the activation of adenosine receptors increased total Hcy levels in the serum. The results obtained clearly demonstrated that NECA increased total Hcy levels in the serum.

2. Materials and Methods

2.1. Reagents

All reagents used were of analytical grade and purchased from Nacalai Tesque Ltd. (Kyoto, Japan) unless otherwise stated. NECA was purchased from Sigma-Aldrich Co. (Tokyo, Japan). [α-^{32}P]dCTP was purchased from PerkinElmer Japan Co., Ltd. (Yokohama, Japan). Tris(2-carboxyethyl)phosphine (TCEP) and 4-fluoro-7-sul-

fonbenzofurazan, ammonium salt (SBD-F) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

2.2. Animal Treatments

Male mice (*ddY* strain, 4 weeks old, 20 - 25 g) were purchased from Japan SLC and maintained for a 1-week acclimation period prior to the start of the experiments under the following conditions: laboratory food (MF, Oriental Yeast Co., Ltd., Japan) and water were provided *ad libitum*, with a 12-h light: dark cycle (8:00 to 20:00 light/20:00 to 8:00 dark). In all experiments, food was withheld from the animals starting at 18:00 for 16 h prior to the administration of NECA. Seven experiments were performed using the NECA treatment. Details of the NECA treatment methods have been described previously [19]. Briefly, after the fasting period, mice were intraperitoneally (i.p.) administered NECA (0.1 mg/kg in 1 mL saline/100 g body weight: NECA0.1 group, or 0.3 mg/kg in 1 mL saline/100 g body weight: NECA0.3 group) and blood and livers were collected 1, 3, 6, or 10 h later under ether anesthesia. In all experiments, control mice were administered an equivalent volume of saline (1 mL saline per 100 g body weight). In all experiments, blood from the descending aorta was obtained under ether anesthesia, and allowed to clot for 15 min at room temperature and a further 30 min at 4˚C. Serum was then prepared by centrifugation at 5000 rpm for 10 min and stored at −80˚C for later analyses. Tissue samples were collected, immediately frozen in liquid nitrogen, and stored at −80˚C until analyzed. All animal experiments were performed in accordance with the Kobe Gakuin University Guidelines that were approved by the Committee on the Ethics of Animal Experiments in that institution.

2.3. RNA Preparation and Hybridization Analysis

Total RNA was extracted using the acid guanidine thiocyanate-phenol-chloroform method [20]. Details of the northern hybridization method have been described previously [23]. The liver-specific *MAT*1*A* gene has two transcripts due to the presence of two promoters for this gene. The expression of the main transcript was detected with the *MAT*1*A*1*st* probe. A 3'-noncoding region fragment was used as a non-liver-type methionine adenosyltransferase 2A (*MAT*2*A*)-specific probe because the *MAT*2*A* sequence of the coding region was similar to that of *MAT*1*A*. The *MAT*1*A*1*st* fragment [24], *MAT*2*A-specific* fragment (1673 to 1868 bp of cDNA fragment GenBank accession No. AB070266), *GNMT* fragment (251 to 779 bp of cDNA fragment GenBank accession No. BC014283), *CBS* fragment (297 to 1099 bp of cDNA fragment GenBank accession No. BC 013472), *BHMT* fragment (1409 to 1854 bp of cDNA fragment GenBank accession No. NM_016668), and 18S rRNA fragment were labeled using [α-^{32}P]dCTP and a Random Primer DNA Labeling Kit Ver. 2.0 (Takara Shuzo, Japan) and then used as probes for northern hybridization.

2.4. Measurement of Methionine Metabolites

AdoMet and AdoHcy levels in the liver were measured using an HPLC method [25] and total GSH in the liver was measured using a microtiter plate assay [26], as described previously [23]. Total Hcy and total cysteine levels (total Cys: free and protein-bound cysteine) in the serum were measured using an HPLC method [27]. Briefly, a mixture of 50 μL of serum, 25 μL of an internal standard, and 25 μL of phosphate-buffered saline (PBS, pH 7.4) was incubated with 10 μL of 100 mg/mL TCEP for 30 min at room temperature in order to reduce and release protein-bound thiols. After this incubation, 90 μL of 100 mg/mL trichloroacetic acid containing 1 mmol/L EDTA was added for deproteinization, centrifuged at 15,000 ×g for 10 min, and 50 μL of the supernatant was added to a tube containing 10 μL of 1.55 mol/L NaOH; 125 μL of 0.125 mol/L borate buffer containing 4 mmol/L EDTA, pH 9.5; and 50 μL of 1 mg/mL SBD-F in the borate buffer. The sample was then incubated for 60 min at 60˚C. HPLC was performed on a Waters M-600 pump equipped with a Waters 2475 Multi λ Fluorescence Detector (385 nm excitation, 515 nm emission). The separation of SBD-derivatized thiols was performed on a μ-BONDASPHERE C18 column (Waters, 5 μm, 100 A, 150 × 3.9 mm) with a 20-μL injection volume and 0.1 mol/L acetate buffer, pH 5.5, containing 30 ml/L methanol as the mobile phase at a flow rate of 1.0 mL/min and column temperature of 29˚C.

2.5. Statistical Analysis

All data are expressed as the mean ± the standard error of the mean (SEM). The unpaired Student's *t*-test was

used to compare NECA-treated groups to the control groups. All statistical analyses were performed using the statistical software package Prism Ver. 5.0 (GraphPad Software, Inc., USA).

3. Results

3.1. Effects of NECA on Total Hcy and Total Cys Levels in the Serum

As shown in **Table 1**, serum total Hcy and total Cys levels significantly increased after 16 h of fasting. The administration of a low dose of NECA (NECA0.1 group) to mice fasted for 16 h resulted in higher serum total Hcy levels than those in the control group at 1 h (Experiment 1). Serum total Hcy levels were also significantly elevated at 3 h (Experiment 2), but were not significantly different from those in the control group at 6 h (Experiment 3). The administration of a high dose of NECA (NECA0.3 group) resulted in significantly higher serum total Hcy levels than those in the control group at 1 h, 3 h, 6 h, and 10 h (Experiments 4, 5, 6, and 7), gradually increasing Hcy levels to 19.7 μM. The effects of NECA on serum total Cys levels were the same as those on total Hcy levels.

Table 1. Effects of NECA on the content of total homocysteine and total cysteine in the serum.

Groups	Serum	
	Total Hcy	Total Cys
	(μmol/L)	(μmol/L)
Normal (n = 6)	4.73 ± 0.17	182 ± 9.8
Fast (n = 6)	6.27 ± 0.28**	252 ± 9.0**
Experiment 1		
Control (1 h) (n = 5)	4.35 ± 0.35	170 ± 7.8
NECA0.1 (1 h) (n = 5)	5.75 ± 0.40*	271 ± 11.3**
Experiment 2		
Control (3 h) (n = 8)	6.26 ± 0.45	192 ± 16.9
NECA0.1 (3 h) (n = 9)	9.30 ± 1.04*	277 ± 27.6*
Experiment 3		
Control (6 h) (n = 4)	7.44 ± 0.27	149 ± 6.4
NECA0.1 (6 h) (n = 5)	9.18 ± 1.82	175 ± 20.9
Experiment 4		
Control (1 h) (n = 5)	4.14 ± 0.36	179 ± 10.5
NECA0.3 (1 h) (n = 5)	6.60 ± 0.51**	308 ± 18.0**
Experiment 5		
Control (3 h) (n = 4)	7.19 ± 0.45	157 ± 20.0
NECA0.3 (3 h) (n = 4)	11.5 ± 0.98**	279 ± 21.6**
Experiment 6		
Control (6 h) (n = 6)	5.61 ± 0.11	243 ± 10.3
NECA0.3 (6 h) (n = 7)	14.8 ± 0.49**	487 ± 22.0**
Experiment 7		
Control (10 h) (n = 7)	7.41 ± 0.47	253 ± 7.5
NECA0.3 (10 h) (n = 8)	19.7 ± 0.95**	494 ± 47.4**

Mice were administered (i.p.) saline (1 mL/100g body weight; control), 0.1 mg/kg or 0.3 mg/kg NECA. Data are presented as the mean ± SEM. Unpaired Student's *t*-tests were used to compare NECA-treated groups to the control groups. *$p < 0.05$, **$p < 0.01$: significantly different from each control.

3.2. Effects of NECA on Other Methionine Metabolite Levels in the Liver

We previously reported that fasting for 16 h decreased AdoMet and GSH levels, and increased AdoHcy levels in the livers of mice [23]. In the present study, as shown in **Table 2**, the administration of a low dose of NECA (NECA0.1 group) to mice fasted for 16 h resulted in lower liver GSH levels than those in the control group at 1 h (Experiment 1). Liver GSH levels were also significantly lower at 3 h (Experiment 2), while GSH levels were not significantly different from those in the control group at 6 h (Experiment 3). The administration of a high dose of NECA (NECA0.3 group) resulted in liver GSH levels that were significantly lower than those in the control group at 1 h, 6 h, and 10 h (Experiments 4, 6, and 7). The effects of NECA on total Hcy levels in the serum and GSH levels in the liver were similar at each dose and time. Furthermore, the low and high doses of NECA both led to significantly higher AdoMet levels than those in the control group at 1 h (Experiments 1 and 4). AdoMet levels at 3 h, 6 h, and 10 h were not significantly different from those in the control group (Experiments 2, 3, 5, 6, and 7). AdoHcy levels were significantly lower in the NECA0.3 group than in the control group 6 h and 10 h after the administration of NECA (Experiments 6 and 7), while the administration of a low dose of NECA had less of an impact on AdoHcy levels.

Table 2. Effects of NECA on the content of methionine metabolites in the liver.

Groups	Liver		
	AdoMet	AdoHcy	Total GSH
	(nmol/g)	(nmol/g)	(μmol/g)
Experiment 1			
Control (1 h) (n = 5)	68.4 ± 7.6	28.7 ± 4.7	3.29 ± 0.09
NECA0.1 (1 h) (n = 5)	100 ± 6.5*	13.6 ± 2.6*	2.27 ± 0.19**
Experiment 2			
Control (3 h) (n = 8)	49.3 ± 7.3	20.8 ± 6.6	2.72 ± 0.16
NECA0.1 (3 h) (n = 9)	49.4 ± 3.7	12.2 ± 2.9	1.88 ± 0.14**
Experiment 3			
Control (6 h) (n = 4)	46.3 ± 13.2	28.5 ± 6.7	2.79 ± 0.11
NECA0.1 (6 h) (n = 5)	46.7 ± 9.8	16.0 ± 8.8	2.72 ± 0.27
Experiment 4			
Control (1 h) (n = 5)	79.0 ± 4.3	15.2 ± 3.1	3.08 ± 0.15
NECA0.3 (1 h) (n = 5)	115 ± 8.7**	20.3 ± 3.1	2.46 ± 0.09**
Experiment 5			
Control (3 h) (n = 4)	52.8 ± 9.0	27.7 ± 8.4	2.58 ± 0.27
NECA0.3 (3 h) (n = 4)	55.4 ± 5.7	9.4 ± 4.4	2.10 ± 0.11
Experiment 6			
Control (6 h) (n = 6)	71.2 ± 12.7	20.7 ± 3.7	1.81 ± 0.10
NECA0.3 (6 h) (n = 7)	72.1 ± 8.0	7.6 ± 2.0**	1.13 ± 0.07**
Experiment 7			
Control (10 h) (n = 6)	65.0 ± 6.9	23.9 ± 2.8	3.29 ± 0.29
NECA0.3 (10 h) (n = 7)	72.9 ± 4.8	7.9 ± 3.8**	2.24 ± 0.13**

Mice were administered (i.p.) saline (1 mL/100g body weight; control), 0.1 mg/kg or 0.3 mg/kg NECA. Data are presented as the mean ± SEM. Unpaired Student's *t*-tests were used to compare the NECA-treated groups to the control groups. $^{*}p < 0.05$, $^{**}p < 0.01$: significantly different from each control.

3.3. Effects of NECA on mRNA Expression of Methionine Cycle Enzymes in the Liver

Figure 1 shows changes in the mRNA expression of methionine cycle enzymes in Experiments 4, 5, and 6. The expression of methionine cycle enzymes did not significantly change 1 h after the administration of NECA. The expression of *MAT*1*A* mRNA was significantly decreased in the liver 6 h after the NECA treatment, while that of *MAT*2*A* was increased. The changes observed in the expression of MAT in the present study were consistent with previous findings obtained in ischemic livers [18] or with liver regeneration [28]. The expression of GNMT, which eliminates excess AdoMet, was significantly decreased 6 h after the NECA treatment. The expression of CBS, which converts Hcy to cystathionine through the transsulfuration pathway, and BHMT, which converts Hcy to methionine, was also decreased at 6 h.

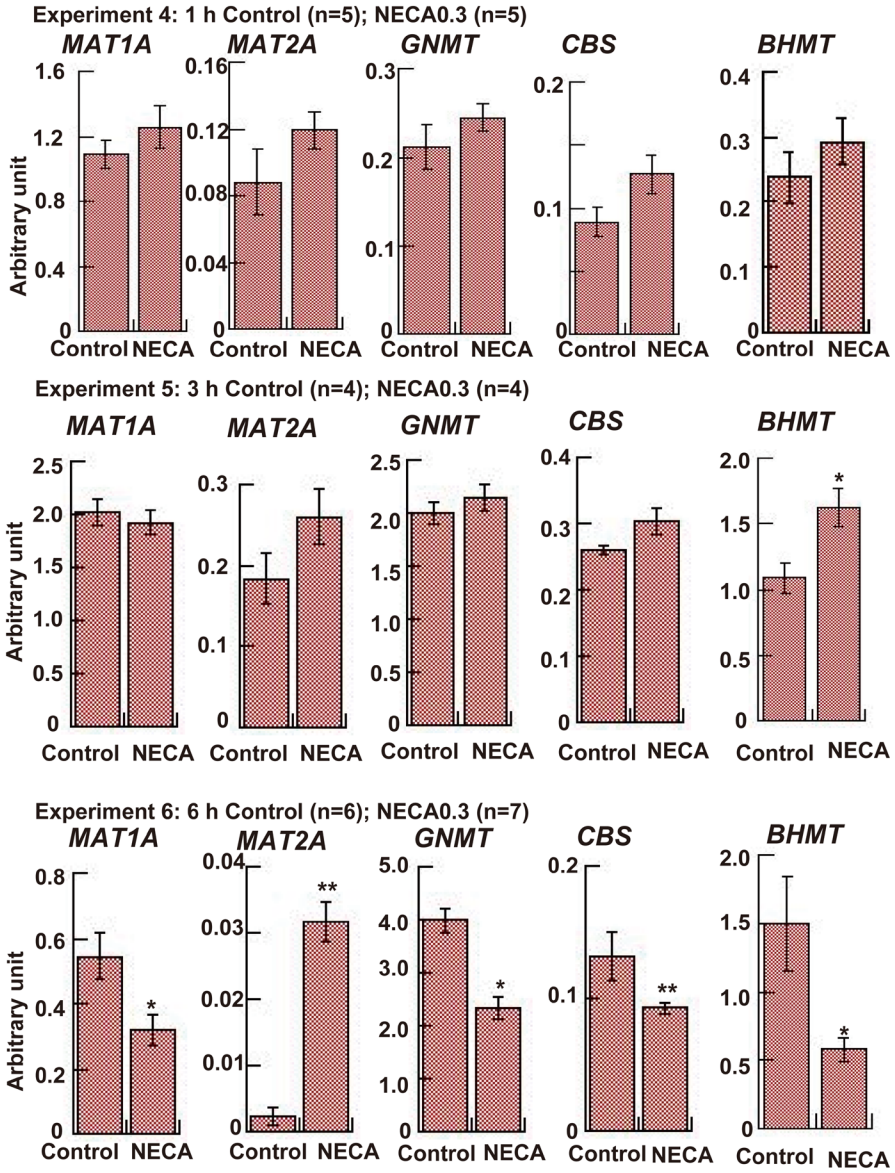

Figure 1. Effects of NECA on the mRNA expression of methionine cycle enzymes in the mouse liver. Northern hybridization was performed on the liver RNA of mice in experiments 4, 5, and 6. The mean ± SEM of the ratio of each enzyme mRNA to the level of the 18S rRNA signal is shown as an arbitrary unit. Unpaired Student's *t*-tests were used to compare NECA-treated groups with the control groups. $^{*}p < 0.05$, $^{**}p < 0.01$: significantly different from each control.

4. Discussion

In the present study, an increase in total Hcy levels and AdoMet levels, and decrease in GSH levels occurred 1 h after the NECA treatment. These results were not due to changes in the expression of methionine metabolic enzymes, which remained unchanged 1 h after the NECA treatment (**Figure 1**). The effects of NECA on methionine metabolism are summarized in **Figure 2**. No previous study has demonstrated that adenosine has the ability to directly affect CBS; however, the overproduction of carbon monoxide (CO), which is generated by heme oxygenase (HO), is found to inhibit transsulfuration [11]. CO has been shown to inhibit CBS activity and increase AdoMet concentrations [11]. Adenosine and NECA were previously reported to markedly induce HO in macrophages [29]. Hcy, which is a substrate of CBS, may be increased by NECA via the CO-induced inhibition of CBS, and GSH may be decreased by the CO-induced inhibition of transsulfuration. However, the mechanism by which NECA affects transsulfuration in the short term has not yet been elucidated.

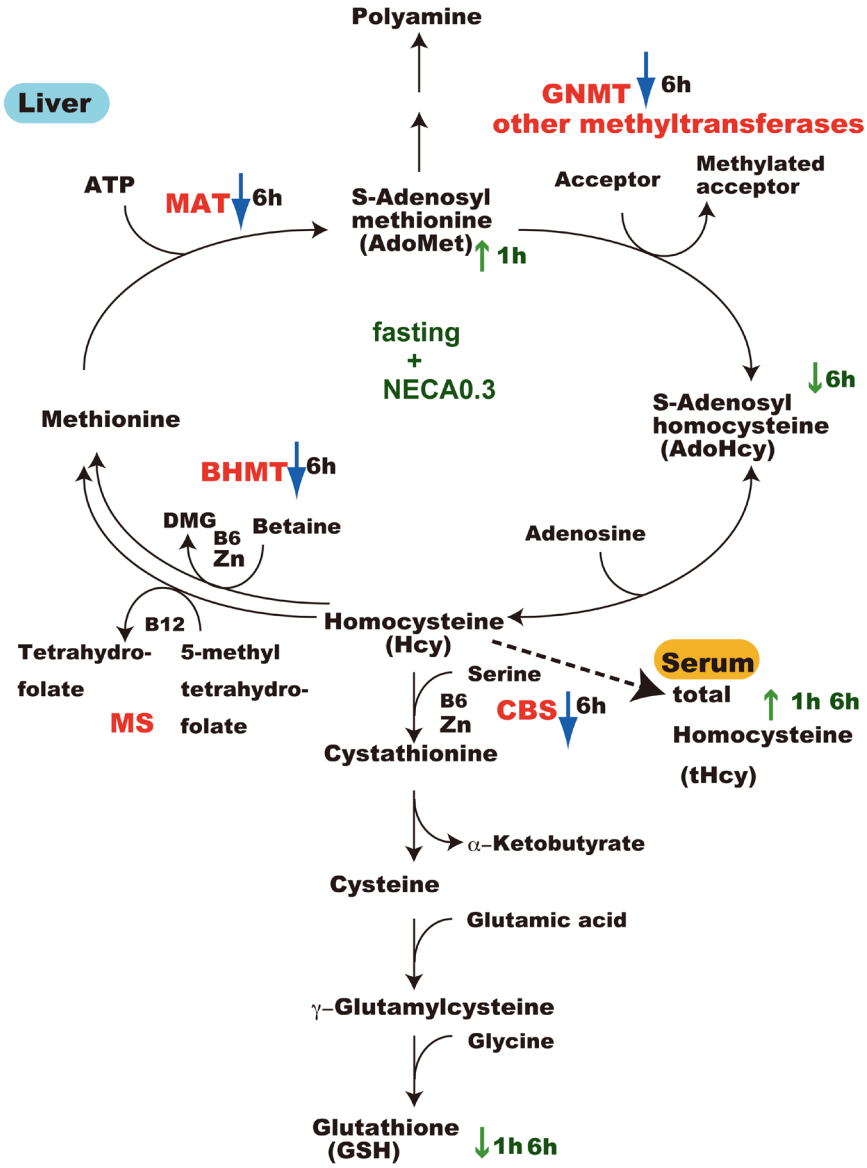

Figure 2. Effects of NECA on the methionine metabolic pathway. MAT: methionine adenosyltransferase, GNMT: glycine *N*-methyltransferase, CBS: cystathionine *β*-synthase, BHMT: betaine-homocysteine *S*-methyltransferase, MS: methionine synthase (Map is based on Sakata SF 2005).

GSH was maintained at a low level for up to 10 h by the NECA0.3 treatment and transsulfuration may have been continuously inhibited by the NECA0.3 treatment. Total Hcy levels were also continuously increased for up to 10 h by the NECA0.3 treatment, and decreased AdoHcy levels were observed 6 h and 10 h after the NECA0.3 treatment. Long-term elevations in serum total Hcy levels by NECA may be maintained by attenuating the expression of methionine metabolic enzymes via the following mechanisms: The expression of methionine metabolic enzymes in the liver was reduced 6 h after the NECA0.3 treatment (**Figure 1**); the flow of the methionine cycle may have been decreased by changes in the expression of MAT (decreased liver-specific *MAT*1*A* expression and increased non-liver type *MAT*2*A* expression) because MATIII (Km for methionine: 215 μM - 7 mM) is the true liver-specific isoform responsible for methionine metabolism [30] and the generation rate of AdoMet by MATII (non-liver type enzyme) was modest with a low Km (80 μM for methionine) [31]; inhibition of the methyltransferases, BHMT [32] and GNMT [33], induces hyperhomocysteinemia; decreases in AdoHcy levels may be caused by reductions in methyltransferase levels. However, the mechanisms by which NECA continuously increased total Hcy levels have not yet been elucidated in detail.

5. Conclusion

The present study confirmed that the non-specific adenosine receptor agonist NECA continuously increased total Hcy levels in the serum. The inhibition of adenosine receptors may decrease the risk of cardiovascular diseases because an increase in serum total Hcy levels is a known risk factor.

Conflict of Interest

There are no conflicts of interest to declare.

References

[1] Antoniades, C., Antonopoulos, A.S., Tousoulis, D., Marinou, K. and Stefanadis, C. (2009) Homocysteine and Coronary Atherosclerosis: from Folate Fortification to the Recent Clinical Trials. *European Heart Journal*, **30**, 6-15. http://dx.doi.org/10.1093/eurheartj/ehn515

[2] Refsum, H., Ueland, P.M., Nygard, O. and Vollset, S.E. (1998) Homocysteine and Cardiovascular Disease. *Annual Review of Medicine*, **49**, 31-62. http://dx.doi.org/10.1146/annurev.med.49.1.31

[3] Garcia-Tevijano, E.R., Berasain, C., Rodriguez, J.A., Corrales, F.J., Arias, R., Martin-Duce, A., Caballeria, J., Mato, J.M. and Avila, M.A. (2001) Hyperhomocysteinemia in Liver Cirrhosis: Mechanisms and Role in Vascular and Hepatic Fibrosis. *Hypertension*, **38**, 1217-1221. http://dx.doi.org/10.1161/hy1101.099499

[4] Araki, A., Ito, H., Majima, Y., Hosoi, T. and Orimo, H. (2003) Association between Plasma Homocysteine Concentrations and Asymptomatic Cerebral Infarction or Leukoaraiosis in Elderly Diabetic Patients. *Geriatrics & Gerontology International*, **3**, 15-23. http://dx.doi.org/10.1046/j.1444-1586.2003.00051.x

[5] Elanchezhian, R., Palsamy, P., Madson, C.J., Lynch, D.W. and Shinohara, T. (2012) Age-Related Cataracts: Homocysteine Coupled Endoplasmic Reticulum Stress and Suppression of Nrf2-Dependent Antioxidant Protection. *Chemico-Biological Interactions*, **200**, 1-10. http://dx.doi.org/10.1016/j.cbi.2012.08.017

[6] Mudd, S.H., Finkelstein, J.D., Refsum, H., Ueland, P.M., Malinow, M.R., Lentz, S.R., Jacobsen, D.W., Brattstrom, L., Wilcken, B., Wilcken, D.E., Blom, H.J., Stabler, S.P., Allen, R.H., Selhub, J. and Rosenberg, I.H. (2000) Homocysteine and Its Disulfide Derivatives: A Suggested Consensus Terminology. *Arteriosclerosis Thrombosis and Vascular Biology*, **20**, 1704-1706. http://dx.doi.org/10.1161/01.ATV.20.7.1704

[7] Finkelstein, J.D. (1990) Methionine Metabolism in Mammals. *The Journal of Nutritional Biochemistry*, **1**, 228-237. http://dx.doi.org/10.1016/0955-2863(90)90070-2

[8] Stipanuk, M.H. (2004) Sulfur Amino Acid Metabolism: Pathways for Production and Removal of Homocysteine and Cysteine. *Annual Review of Nutrition*, **24**, 539-577. http://dx.doi.org/10.1146/annurev.nutr.24.012003.132418

[9] Chou, J.Y. (2000) Molecular Genetics of Hepatic Methionine Adenosyltransferase Deficiency. *Pharmacology & Therapeutics*, **85**, 1-9. http://dx.doi.org/10.1016/s0163-7258(99)00047-9

[10] De La Haba, G. and Cantoni, G.L. (1959) The Enzymatic Synthesis of *S*-Adenosyl-L-Homocysteine from Adenosine and Homocysteine. *The Journal of Biological Chemistry*, **234**, 603-608. http://www.jbc.org/content/234/3/603.short

[11] Shintani, T., Iwabuchi, T., Soga, T., Kato, Y., Yamamoto, T., Takano, N., Hishiki, T., Ueno, Y., Ikeda, S., Sakuragawa, T., Ishikawa, K., Goda, N., Kitagawa, Y., Kajimura, M., Matsumoto, K. and Suematsu, M. (2009) Cystathionine Beta-Synthase as a Carbon Monoxide-Sensitive Regulator of Bile Excretion. *Hepatology*, **49**, 141-150. http://dx.doi.org/10.1002/hep.22604

[12] Purohit, V., Abdelmalek, M.F., Barve, S., Benevenga, N.J., Halsted, C.H., Kaplowitz, N., Kharbanda, K.K., Liu, Q.Y., Lu, S.C., McClain, C.J., Swanson, C. and Zakhari, S. (2007) Role of *S*-Adenosylmethionine, Folate, and Betaine in the Treatment of Alcoholic Liver Disease: Summary of a Symposium. *The American Journal of Clinical Nutrition*, **86**, 14-24. http://ajcn.nutrition.org/content/86/1/14.full

[13] Mudd, S.H. and Poole, J.R. (1975) Labile Methyl Balances for Normal Humans on Various Dietary Regimens. *Metabolism*, **24**, 721-735. http://dx.doi.org/10.1016/0026-0495(75)90040-2

[14] Bontemps, F., Vincent, M.F. and Van den Berghe, G. (1993) Mechanisms of Elevation of Adenosine Levels in Anoxic Hepatocytes. *Biochemical Journal*, **290**, 671-677. http://www.biochemj.org/content/290/3/671 http://dx.doi.org/10.1042/bj2900671

[15] Fredholm, B.B. (2007) Adenosine, an Endogenous Distress Signal, Modulates Tissue Damage and Repair. *Cell Death and Differentiation*, **14**, 1315-1323. http://dx.doi.org/10.1038/sj.cdd.4402132

[16] Fredholm, B.B., Irenius, E., Kull, B. and Schulte, G. (2001) Comparison of The Potency of Adenosine as an Agonist at Human Adenosine Receptors Expressed in Chinese Hamster Ovary Cells. *Biochemical Pharmacology*, **61**, 443-448. http://dx.doi.org/10.1016/S0006-2952(00)00570-0

[17] Fredholm, B.B., Ijzerman, A.P., Jacobson, K.A., Klotz, K.N. and Linden, J. (2001) International Union of Pharmacology. XXV. Nomenclature and Classification of Adenosine Receptors. *Pharmacological Reviews*, **53**, 527-552. http://pharmrev.aspetjournals.org/content/53/4/527.full.pdf+html

[18] Ito, K., Miwa, N., Hagiwara, K., Yano, T., Shimizu-Saito, K., Goseki, N., Iwai, T. and Horikawa, S. (1999) Regulation of Methionine Adenosyltransferase Activity by the Glutathione Level in Rat Liver during Ischemia-Reperfusion. *Surgery Today*, **29**, 1053-1058. http://dx.doi.org/10.1007/PL00010035

[19] Matsuda, K., Horikawa, Y., Sasaki, Y. and Sakata, S.F. (2014) The Adenosine Receptor Agonist 5'-*N*-Ethylcarboxamide-Adenosine Increases Glucose 6-Phosphatase Expression and Gluconeogenesis. *Pharmacology & Pharmacy*, **5**, 15-23. http://dx.doi.org/10.4236/pp.2014.51004

[20] Sakata, S.F., Fujino, M., Matsuda, K., Maeda, M., Ohira, H., Kawasaki, K. and Tamaki, N. (2007) Mechanism of Liver Tyrosine Aminotransferase Increase in Ethanol-Treated Mice and Its Effect on Serum Tyrosine Level. *Journal of Nutritional Science & Vitaminology*, **53**, 489-495. http://doi.org/10.3177/jnsv.53.489

[21] Marston, H.M., Finlayson, K., Maemoto, T., Olverman, H.J., Akahane, A., Sharkey, J. and Butcher, S.P. (1998) Pharmacological Characterization of a Simple Behavioral Response Mediated Selectively by Central Adenosine A1 Receptors, Using *in Vivo* and *in Vitro* Techniques. *Journal of Pharmacology and Experimental Therapeutics*, **285**, 1023-1030. http://jpet.aspetjournals.org/content/285/3/1023.long

[22] Chomczynski, P. and Sacchi, N. (1987) Single-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction. *Analytical Biochemistry*, **162**, 156-159. http://www.sciencedirect.com/science/article/pii/0003269787900212

[23] Sakata, S.F., Okumura, S., Matsuda, K., Horikawa, Y., Maeda, M., Kawasaki, K., Chou, J.Y. and Tamaki, N. (2005) Effect of Fasting on Methionine Adenosyltransferase Expression and the Methionine Cycle in the Mouse Liver. *Journal of Nutritional Science & Vitaminology*, **51**, 118-123. http://doi.org/10.3177/jnsv.51.118

[24] Sakata, S.F., Tamaoka, K., Matsuda, K., Kaneko, M., Chou, J.Y. and Tamaki, N. (1998) Effect of Glucocorticoids on the Mouse Methionine Adenosyltransferase A1 Gene Expression, Which Is Regulated by Two Promoters. *Biochimica et Biophysica Acta*, **1442**, 127-136. http://dx.doi.org/10.1016/S0167-4781(98)00156-0

[25] She, Q.B., Nagao, I., Hayakawa, T. and Tsuge, H. (1994) A Simple HPLC Method for the Determination of *S*-Adenosylmethionine and *S*-Adenosylhomocysteine in Rat Tissues: The Effect of Vitamin B6 Deficiency on These Concentrations in Rat Liver. *Biochemical and Biophysical Research Communications*, **205**, 1748-1754. http://dx.doi.org/10.1006/bbrc.1994.2871

[26] Baker, M.A., Cerniglia, G.J. and Zaman, A. (1990) Microtiter Plate Assay for the Measurement of Glutathione and Glutathione Disulfide in Large Numbers of Biological Samples. *Analytical Biochemistry*, **190**, 360-365. http://www.sciencedirect.com/science/article/pii/000326979090208Q http://dx.doi.org/10.1016/0003-2697(90)90208-Q

[27] Pfeiffer, C.M., Huff, D.L. and Gunter, E.W. (1999) Rapid and Accurate HPLC Assay for Plasma Total Homocysteine and Cysteine in a Clinical Laboratory Setting. *Clinical Chemistry*, **45**, 290-292. http://www.clinchem.org/content/45/2/290.long

[28] Mato, J.M., Alvarez, L., Ortiz, P. and Pajares, M.A. (1997) *S*-Adenosylmethionine Synthesis: Molecular Mechanisms and Clinical Implications. *Pharmacology & Therapeutics*, **73**, 265-280. http://dx.doi.org/10.1016/S0163-7258(96)00197-0

[29] Haschemi, A., Wagner, O., Marculescu, R., Wegiel, B., Robson, S.C., Gagliani, N., Gallo, D., Chen, J.F., Bach, F.H. and Otterbein, L.E. (2007) Cross-Regulation of Carbon Monoxide and The Adenosine A2a Receptor in Macrophages. *The Journal of Immunology*, **178**, 5921-5929. http://dx.doi.org/10.4049/ jimmunol.178.9.5921

[30] del Pino, M.M., Corrales, F.J. and Mato, J.M. (2000) Hysteretic Behavior of Methionine Adenosyltransferase III. Methionine Switches between Two Conformations of the Enzyme with Different Specific Activity. *The Journal of Biological Chemistry*, **275**, 23476-23482. http://dx.doi.org/10.1074/jbc.M002730200

[31] Mitsui, K., Teraoka, H. and Tsukada, K. (1988) Complete Purification and Immunochemical Analysis of *S*-Adenosylmethionine Synthetase from Bovine Brain. *The Journal of Biological Chemistry*, **263**, 11211-11216. http://www.jbc.org/content/263/23/11211.abstract?sid=4685db2a-5380-481d-a9c1-b31235c588f4

[32] Collinsova, M., Strakova, J., Jiracek, J. and Garrow, T.A. (2006) Inhibition of Betaine-Homocysteine *S*-Methyltransferase Causes Hyperhomocysteinemia in Mice. *The Journal of Nutrition*, **136**, 1493-1497. http://jn.nutrition.org/content/136/6/1493

[33] Tanghe, K.A., Garrow, T.A. and Schalinske, K.L. (2004) Triiodothyronine Treatment Attenuates the Induction of Hepatic Glycine *N*-Methyltransferase by Retinoic Acid and Elevates Plasma Homocysteine Concentrations in Rats. *The Journal of Nutrition*, **134**, 2913-2918. http://jn.nutrition.org/content/134/11/2913.abstract