



# The Effects of Certain Gasotransmitters Inhibition on Homocysteine Acutely Induced Changes on Rat Cardiac Acetylcholinesterase Activity

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## ABSTRACT

**Background/Aim:** Hyperhomocysteinaemia is linked to higher level of acetylcholinesterase (AChE) in brain, but there is insufficient information on influence of homocysteine (Hcy) and gasotransmitters on cardiac AChE. Thus, the aim of this study was to evaluate the influence of certain gasotransmitter inhibitors in Hcy-induced changes on rat cardiac AChE activity.

**Methods:** Research was performed on 72 male Wistar albino rats distributed into 6 groups: 1) Control group – saline (1 ml 0.9 % NaCl ip); 2) DL-Hcy (8 mmol/kg ip DL homocysteine (DL-Hcy)); 3) L-NAME (10 mg/kg ip N<sup>ω</sup>-Nitro-L-arginine methyl ester (L-NAME), inhibitor of NO production); 4) DL-PAG (50 mg/kg ip DL-propargylglycine (DL-PAG), inhibitor of H<sub>2</sub>S production); 5) DL-Hcy+L-NAME (8 mmol/kg ip DL-Hcy + 10 mg/kg ip L-NAME); and 6) DL-Hcy+DL-PAG (8 mmol/kg ip DL-Hcy + 50 mg/kg ip DL-PAG). All tested substances were administered in a single dose, intraperitoneally, 60 minutes before animals' sacrifice. AChE activity was measured in the rats' cardiac tissue homogenate.

**Results:** Administration of Hcy and L-NAME induced significant decrease in AChE activity compared with control condition. Administration of DL-PAG, DL-Hcy+L-NAME and DL-Hcy+DL-PAG did not change AChE activity compared with the control group.

**Conclusion:** The effects of acute Hcy administration on the cardiac AChE activity are partially mediated via interaction with tested gasotransmitters.

**Key words:** acetylcholinesterase, heart, homocysteine, inhibition.

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## INTRODUCTION

Hyperhomocysteinaemia is an independent predictor of different diseases such as, cardiovascular and cerebrovascular diseases, neurodegeneration and cancer.<sup>1</sup> Increased blood homocysteine (Hcy) level independently predicted all-cause and cardiovascular mortality in the general and especially in the older population,<sup>2</sup> and it has been recognised as a risk factor for cardiovascu-

lar diseases.<sup>3,4</sup> Different studies<sup>5-9</sup> have demonstrated that increased Hcy concentration was correlated with poor prognosis in patients with acute coronary syndrome, but other studies results were contradictory.<sup>8,10-13</sup> Previous study revealed that increased Hcy may be associated with elevated oxidative stress and inhibition of the butyrylcholinesterase (BuChE) activity.<sup>14</sup>

This may result, probably, in cardiovascular diseases and consequently in an increase of the mortality risk.<sup>14</sup> It has been reported that after both, acute and chronic Hcy administration in rats, serum BuChE activity was significantly decreased. Antioxidants, such as vitamins E and C, avoided the decrease of this enzyme activity caused by acute Hcy administration, implying that free radicals are responsible for reducing BuChE activity under conditions of acute hyperhomocysteinaemia.<sup>14</sup> Another study showed that the increased concentration of Hcy in serum decreases the activity of acetylcholinesterase (AChE).<sup>15</sup> Many tissues, and especially the nerve tissue, are rich in AChE and BuChE.<sup>16</sup> BuChE is the most abundant cholinesterase in serum<sup>17</sup>, while AChE is primarily present in membranes of erythrocytes.<sup>18</sup> AChE is an enzyme belonging to the group of serine hydrolase with the primary function of hydrolysing neurotransmitter acetylcholine.<sup>19</sup> It is mainly located at cholinergic brain synapses and neuromuscular junctions. Although less than there, AChE activity could be also very important for functioning of cholinergic system within the heart.<sup>20</sup> Even if it hydrolyses acetylcholine, it is demonstrated that AChE is also present in hematopoietic tissue and cancer cells that are not innervated by cholinergic system.<sup>21</sup>

Cetrain signaling gaseous molecules or gasotransmitters, such as nitric oxide (NO) and hydrogen sulfide (H<sub>2</sub>S) participate in effects of Hcy-thiolactone on the coronary circulation and myocardial function.<sup>22,23</sup> Gasotransmitters have many important roles. They participate in the regulation of inflammation, modulation of mitochondria respiration and activation of antioxidant enzymes and consequently have essential role in oxidative stress regulation, so their cardiac effects are expected and reasonable.<sup>24</sup> It is demonstrated that S-nitroso-Hcy inhibits hydrogen peroxide production with participation of NO.<sup>25</sup> H<sub>2</sub>S lowers plasma Hcy level and it scavenges reactive oxygen species and functions as an antioxidant.<sup>26,27</sup>

However, connection between Hcy and certain gasotransmitter effects is still not fully understood. Taken into consideration assumptions referring to existence of AChE within the heart<sup>20</sup>, as well as insufficient data about complex interaction between gasotransmitters and Hcy in the cardiac muscle, researches in order to evaluate

the role of gasotransmitters (NO and H<sub>2</sub>S) on Hcy-induced effects on AChE in rat heart are necessary. Therefore, the aim of this study was to assess the effects of acute administration of DL-Hcy, as well as administration of DL-Hcy together with specific inhibitors of different gasotransmitters, such as N $\omega$ -nitro-L-arginine methyl ester (L-NAME) and DL-propargyl Glycine (DL-PAG) on AChE activity in rat heart tissue.

## METHODS

### Physiological Assay and Experimental Protocol

Experiment was performed on male Wistar albino rats (n = 72, 12 in each experimental group, 10 weeks old, body weight 250 ± 30 g). Experimental animals were housed in pairs with standard food and water available ad libitum. The ambient conditions were strictly controlled (air temperature of 22±1°C, relative humidity of 50%, and a cycle of light: dark 12:12 hours, starting light period at 8 AM). In all experimental groups, tested substances were administered in a single dose, intraperitoneally (*ip*), 60 minutes before sacrificing of animals. The experimental animals were distributed randomly in one of six groups: 1) Control group – saline (1 ml 0.9% NaCl *ip*, pH 7.4); 2) DL-Hcy group (8 mmol/kg *ip* DL homocysteine); 3) L-NAME group (10 mg/kg *ip* L-NAME as inhibitor of NO production via inhibition of nitric oxide synthase); 4) DL-PAG group (50 mg/kg *ip* DL-PAG as inhibitor of H<sub>2</sub>S production via inhibition of cystathionine gamma lyase); 5) DL-Hcy+L-NAME group (8 mmol/kg *ip* DL-Hcy + 10 mg/kg *ip* L-NAME); 6) DL-Hcy+DL-PAG group (8 mmol/kg *ip* DL-Hcy + 50 mg/kg *ip* DL-PAG).

All experimental procedures were done in agreement with prescribed legislation (EU Directive for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes 86/609/EES) and the principles of ethics.

### Tissue preparation

Sixty minutes after *ip* administration of tested substances, the rats were euthanised by decapitation. Whole rats' hearts were isolated and the blood was stored in test tubes coated in heparin. The hearts were rinsed in cold phosphate buffer pH 8.0, and homogenised in the same buffer.

The final tissue concentration was 20 mg tissue per 1 ml buffer.

### Biochemical analyses

For the biochemical analyses blood was collected through a glass funnel and placed in appropriate vacutainers coated in heparin. After the collection, the samples remained at room temperature for 15 minutes and then they were centrifuged (15 min x 3000 rpm) and in the obtained plasma Hcy concentration was analyzed. Following the sacrificing of rats, AChE activity was determined in samples of cardiac tissue homogenate.

### Determination of plasma Hcy

For this process the samples were analyzed using the electrochemiluminescence method (ECL-electrochemiluminescence immunoassay system, ADVIA Centaur XP System, Siemens Healthcare GmbH, Erlangen, Germany). The reference value for Hcy was  $< 15 \mu\text{mol/l}$ .

### Determination of AChE activity

The specific activity of AChE in samples of cardiac tissue homogenate was determined *in vitro* by method of Ellman. The method is based on reaction of a colouring reagent (5, 5-dithio-bis-2-nitrobenzoic acid, DTNB) with the hydrolysis product of thioholine substrate, acetylcholine iodide (AChI), thioholine, to give the compound 5-thio-2-nitro-benzoate-yellow color, whose intensity is proportional to the specific activity of AChE.<sup>28</sup>

### Chemicals used

All chemicals were of p.a. grade quality and were purchased from Sigma Aldrich (Germany).

### Statistical analyses

One-way analysis of variance (ANOVA), followed by Tukey's Post Hoc Test was used for testing statistical significance after testing normality of parameters distribution. Statistical calculation was done using SPSS computer program (SPSS Inc. Chicago, SAD). Values were presented as mean  $\pm$  SEM.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Determination of Hcy

In the Control group plasma Hcy was  $10.4 \pm 0.6 \mu\text{mol/l}$ , while in all other plasma samples levels

of measured Hcy were higher than  $65 \mu\text{mol/l}$ . These results demonstrate moderate hyperhomocysteinemia ( $30\text{--}100 \mu\text{mol/l}$ ).

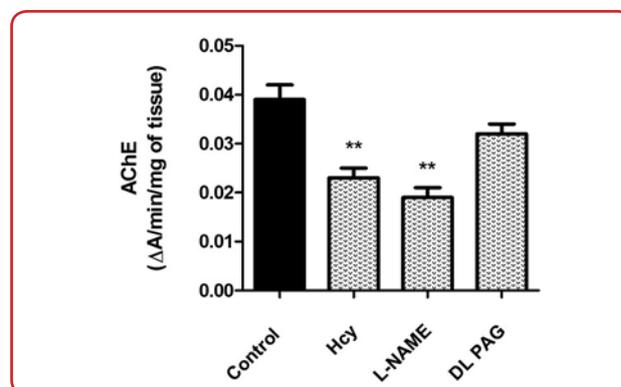


Figure 1 a

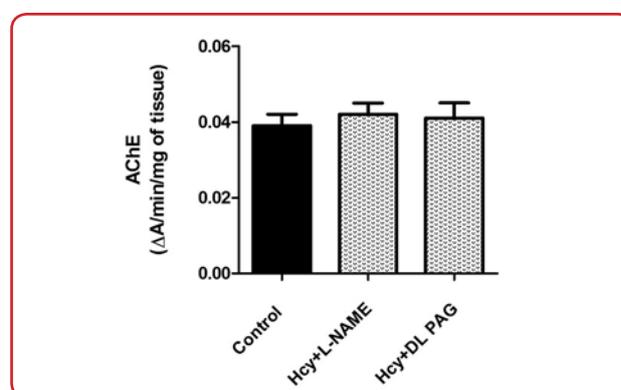


Figure 1 b

### Cardiac tissue homogenate AChE activity

Administration of Hcy ( $0.023 \pm 0.002 \Delta\text{A}/\text{min}/\text{mg}$  of tissue, Figure 1. a) and L-NAME ( $0.019 \pm 0.002 \Delta\text{A}/\text{min}/\text{mg}$  of tissue, Figure 1. a) induced significant decrease in AChE activity compared with the control group ( $0.039 \pm 0.003 \Delta\text{A}/\text{min}/\text{mg}$  of tissue). Administration of DL-PAG ( $0.041 \pm 0.004 \Delta\text{A}/\text{min}/\text{mg}$  of tissue, Figure 1. a), DL-Hcy+L-NAME ( $0.042 \pm 0.003 \Delta\text{A}/\text{min}/\text{mg}$  of tissue, Figure 1. b) and DL-Hcy+DL-PAG ( $0.041 \pm 0.004 \Delta\text{A}/\text{min}/\text{mg}$  of tissue, Figure 1. b) did not induce significant changes in AChE activity compared with control condition.

## DISCUSSION

Few studies have proven certain cardioprotective effects of ACh, but only in conditions of hypoxaemia, ischaemia and inflammation.<sup>29,30</sup> All these conditions are highly correlated with oxidative stress and ROS production. Mentioned studies have shown that these effects are

achieved by cytokine inhibition<sup>30</sup>, but also by activating muscarinic receptors and NO production.<sup>29</sup> This could explain the significant reduction of AChE activity in cardiac tissue, observed in this study, in acutely induced hyperhomocysteinaemia as a compensatory mechanism, leading to increase of ACh. Even more prominent decrease of AChE activity occurred during application of L-NAME, which is also known as a non-selective muscarinic antagonist.<sup>29</sup> Contrary to this point of view, it could be assumed that one of potential mechanisms through which Hcy manifests its pro-arrhythmogenic potential<sup>31</sup> might be decrease of AChE activity, having in mind that AChE is mainly located in region of SA and AV nodules.<sup>32</sup> Additional investigations are needed to determine whether this is a compensatory mechanism for direct effect of Hcy.

Findings of this study are corroborated by other studies that also showed AChE reduction.<sup>15</sup> Stefanello and coworkers, investigated the effects of Hcy (500  $\mu$ M) on other cholinesterase involved in ACh degradation, ie BuChE, and found that Hcy strongly inhibited activity of this esterase in rats, as well.<sup>15</sup> Also, few years later, the same authors examined and compared acute and chronic effects of Hcy on BuChE activity, and these data suggested the inhibitory effects of Hcy also.<sup>14</sup> The previous study demonstrated that the combination of Hcy and ZnPPR IX has led to increased activity of AChE in relation to the control, suggesting that CO is potentially very important gaseous molecule for mediation of Hcy-induced effects on cardiac AChE.<sup>33</sup>

Finally, the limitation of this study could be that determination of certain gasotransmitters effects was not evaluated directly, but indirectly by inhibition of their production. Data on mRNA or protein levels in the cardiac tissue, as well as cellular data would verify the findings that cardiac AChE activity is altered by homocysteine level.

## CONCLUSION

It has been concluded that Hcy may alter function of rat heart in part by reduction of AChE activity; however, there are limited supportive data presented in the manuscript. This is an association study where administration of Hcy *ip* or certain gasotransmitters production inhibi-

tion was associated with up-, or downregulation of acetylcholine. This study can contribute to the clarification of these interactions.

## CONFLICT OF INTEREST

None.

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## AUTHORSHIP STATEMENT

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