FOLIA MEDICA CRACOVIENSIA
Vol. LI, 1–4, 2011: 29–35
PL ISSN 0015-5616

Bogdan Wiliński[1], Jerzy Wiliński[2], Eugeniusz Somogyi[3],
Joanna Piotrowska[3], Marta Góralska[1]

AMLODIPINE AFFECTS ENDOGENOUS HYDROGEN SULFIDE TISSUE CONCENTRATIONS IN DIFFERENT MOUSE ORGANS

Abstract: *Amlodipine affects endogenous hydrogen sulfide tissue concentrations in different mouse organs*

The interactions between calcium channel blockers' action and the endogenous hydrogen sulfide (H_2S) biology are unknown. CBA strain mice were administered intraperitoneally 3 mg/kg b.w. per day or 10 mg/kg b.w. per day of amlodipine. The control group received physiological saline. The measurements of the free H_2S tissue concentrations were performed with Siegel spectrophotometric modified method. There was as significant fall of H_2S level in the brain and the liver in both groups. The lower amlodipine dose increased the H_2S concentrations in the heart and kidneys while the higher one decreased H_2S accumulation in those organs. Our experiment has shown that amlodipine interferes with H_2S biology and affects its tissue concentrations in different mouse tissues.

Key words: hydrogen sulfide, amlodipine, calcium channel antagonist, nitric oxide, mouse
Słowa kluczowe: siarkowodór, amlodypina, antagoniści kanałów wapniowych, tlenek azotu, mysz

INTRODUCTION

Hydrogen sulfide (H_2S) has been identified as a crucial co-regulator of various physiological processes in mammals [1]. The transmitter has been also shown to participate in pathophysiology of different clinical disorders and is assumed to have some therapeutic properties as several pharmaceutical companies are currently under way to introduce H_2S-based agents to treat various diseases [2]. The actions of amlodipine exceed the vasodilatation elicited by the calcium channel blockade and comprise i.a. the interference with cholesterol, antioxidants, nitric oxide (NO) production, smooth muscle cell proliferation and matrix formation [3]. The interactions of calcium channel blockers (CCBs) with the endogenous H_2S are unknown.

THE AIM OF THE STUDY

The aim of the study is to assess the influence of amlodipine on the endogenous tissue H_2S concentrations in mouse brain, heart, liver and kidney.

MATERIAL AND METHODS

Twenty three CBA strain female mice (11–12 weeks old individuals) of approximate 20 g weight were involved in the study. The animals were housed under standard laboratory conditions and had free access to water and food. They were kept at the temperature of 22–24°C with a light/dark cycle of 12 h.

The study design comprised intraperitoneal injections of a solution containing amlodypine in amounts of 3 mg per kg of body weight daily (group D1, n = 7) or 10 mg per kg of body weight daily (group D2, n = 8) for 5 consecutive days at the same time of the day (10:30 am). The third generation L-type CCB amlodipine (Adipine, Polfa-Rzeszów, Poland) was dissolved in a physiological saline. Each administration comprised 0.2 mL of the solution. The control group (n = 8) received intraperitoneally physiological saline in portions of the same volume. The individuals were randomly assigned to each group. The animals tolerated the applied doses of amlodipine well and remained in a good condition till the end of the experiment. The measurements of the free tissue H_2S concentrations were performed by the use of the modified method of Siegel [4, 5]. The study has been performed in accordance with the guidelines for the care and use of laboratory animals accepted by the Bioethical Committee of the Jagiellonian University Medical College (Kraków, Poland).

Two hours after the last drug or physiological saline injection the animals were killed by cervical dislocation, their brains, hearts, livers and kidneys were quickly removed, homogenized with 0.01 mol/l sodium hydroxide (NaOH): the brain tissue in proportion of 1 to 4, the kidney and the liver of 1 to 5 and the heart of 1 to 10 and frozen. Then 50% trichloroacetic acid (TCA) was added (0.5 mL to 2 g of brain or liver samples in tight capsules of 3 mL and 0.25 mL to 1 g of heart or kidney sample in tight capsules of 2 mL), the suspension was shaken and centrifuged. Subsequently, 1.5 mL samples of brain or liver and 0.75 mL samples of heart or kidney supernatant were moved to 2 mL tight capsules with 0.15 mL or 0.075 mL of 0.02 mol/L N,N-dimethyl-p-phenyl-diamine sulfate in 7.2 mol/L hydrochloric acid (HCl), then 0.15 mL or 0.075 mL of 0.03 mol/L iron (III) chloride ($FeCl_3$) in 1.2 mol/L HCl portions were added, respectively. After 20 minutes in darkness the content was shaken for 1 minute with 1 mL of chloroform.

Absorbance was measured at 650 nm with the Varian Cary 100 spectrophotometer. A standard curve was prepared with an iodometrically determined

0.0001 mol/L sodium sulfide (Na_2S) solution. In all groups of the animals four concurrent analyses of each tissue type were performed.

The statistical analysis was performed within the R Environment by the Student's t-test. Statistical significance was considered when $p < 0.05$.

RESULTS

There was a significant fall in the H_2S concentrations in the brain (D1 by 26.5%, D2 by 12.9%) and in the liver (D1 by 15.6%, D2 by 31.9%) as compared to the control group. The lower amlodipine dose increased the H_2S tissue level in the heart (by 19.3%) and in the kidney (by 17.5%), while the higher amlodipine dose decreased the H_2S contain in those organs (by 19.4% in the heart and by 11.6% in the kidney). The free H_2S tissue concentrations' values are presented in the Table 1.

Table 1 — Tabela 1

Hydrogen sulfide (H_2S) tissue concentrations in mouse brain, heart, liver and kidney following the administration of amlodipine in doses of 3 mg/kg b.w. per day or 10 mg/kg b.w. per day (groups D1 and D2 respectively)

Tkankowe stężenie siarkowodoru (H_2S) w mózgu, sercu, wątrobie i nerce myszy po podaniu amlodypiny w dawkach 3 mg/kg masy ciała dziennie lub 10 mg/kg masy ciała dziennie (odpowiednio grupa D1 i D2)

H_2S tissue concentration [μg/g]	Control group (n = 8)	D1 (n = 7)	D2 (n = 8)
Brain	1.47 ± 0.02	1.08 ± 0.02**	1.28 ± 0.03††
Heart	6.89 ± 0.14	8.22 ± 0.15**	5.55 ± 0.09††
Liver	3.92 ± 0.06	3.31 ± 0.04**	2.67 ± 0.04††
Kidney	7.13 ± 0.07	8.38 ± 0.10**	6.30 ± 0.14††

Statistical significance: *$p < 0.05$ for Control vs D1 groups, **$p < 0.01$ for Control vs D1 groups, †$p < 0.05$ for Control vs D2 groups, ††$p < 0.01$ for Control vs D2 groups

DISCUSSION

The positive charge and high lipid affinity of amlodipine result in the drug accumulation in membranes and inhibition of modified low-density lipoproteins (LDLs) aggregation, mediated by electronegative properties of oxidized lipid [6]. Moreover, the dihydropyridine ring structure of amlodipine resides at the same depth as the sterol nucleus which effects in the declined ability of cholesterol to increase membrane width and to aggregate into crystalline-like domains. Elevated cholesterol content in membranes disrupts i.a. the function of Ca^{2+}

and L-arginine transport leading to overproduction of superoxide from oxygen by endothelial nitric oxide synthase (eNOS), what occurs when the NO substrate is lacking [3]. Amlodipine impedes this process and raises NO formation also with kinins' share. As a vasorelaxant the drug elicits changes in shear stress, an important regulator of NO production, stimulating the formation and preventing the breakdown of kinins by activating and releasing kallikrein [7]. Furthermore, amlodipine's R-enantiomer does not interact with the L-type calcium channels but activates either angiotensin II receptors, or receptors that are closely related to them, stemming in augmented local kinin formation [8].

Amlodipine is a potent inhibitor of vascular smooth muscle cells proliferation. The mechanisms involve Ca^{2+} signaling resulting in depressed extracellular signal-regulated protein kinases activation (ERK1/2, MAPK), inhibition of DNA synthesis and expression of early growth response genes including c-*myc*, c-*fos*, c-*jun* [9, 10]. Amlodipine exerts other antioxidant activities shown to be independent of calcium channel modulation and attributed to proton-donating and resonance-stabilization mechanisms that suppress free-radical reactions [11]. Oxidized LDLs stimulate vascular smooth muscle cells growth through activation of Ras/Raf/MEK/MAPK signaling pathway by a *Pertussis* toxin-sensitive G-protein coupled receptor, whereas amlodipine reduce the LDL oxidation process [3, 12]. Amlodipine was also shown to inhibit metalloproteinases' (MMPs) activity [13].

H_2S is formed from L-cysteine in several enzymatic reactions catalyzed by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST), and non-enzymatic pathways in many tissues [14]. Cytoplasmatic bound sulfur is postulated to absorb and store exogenously applied and endogenously produced H_2S and release it in certain physiologic conditions [15]. H_2S is lipophilic, freely permeates plasma membranes and participates in the sulfhydration of numerous proteins which alters its function. Sulfhydration poses an important physiologic signal and a prominent post-translational modification [16]. The action of H_2S comprises numerous intracellular mechanisms including i.a. adenosine triphosphate (ATP)-sensitive potassium channels (K_{ATP}) stimulation, maintaining protein –SH groups in the reduced state, reaction with reactive oxygen and nitrogen species (ROS and RNS) — protection of proteins and lipids from ROS/RNS-mediated damages, stimulation of cysteine transport to the cell and reduced glutathione (GSH) synthesis, metabolic inhibition, inhibition of L-type calcium channels, an influence on ERKs, phosphoinositide 3'-kinase (PI3K)/Akt (protein kinase B), protein kinase C (PKC) and NF-E2-related factor-2 (Nrf-2) [17–22]. H_2S also exerts some anti-inflammatory effects in certain conditions and normalizes MMP-2 and MMP-9 activity, reduces the NF-κB complex activation and leads to fall in some pro-inflammatory cytokines [23–26]. Moreover, H_2S interacts with carbon monoxide (CO) and NO in a number of ways [27].

As we have demonstrated amlodipine in its biology interferes with the endogenous H_2S, resulting in its altered tissue levels. The effects are dose-dependent and specific for each tissue what is a result of different metabolism, paracrine regulation and various transmitters interactions in organs. Some molecular aspects of amlodipine and H_2S are common like L-calcium channel, NO, ERKs involvement, antioxidant properties or MMPs activity regulation. Experimental data of anti-atherosclerotic, hypotensive and anti-proliferative effects connect both compounds [3, 17]. The interaction mechanisms remain obscure with probable NO involvement as one of the link between CCB and endogenous sulfur biology. The H_2S engagement makes the CCBs' biology even more complex, pondering H_2S multidirectional actions. So far it is unknown whether H_2S mediates any of amlodipine's effects. Interestingly, H_2S bioavailability is affected by other drugs like aspirin, angiotensin-converting enzyme inhibitor ramipril, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) inhibitor atorvastatin and paracetamol [28–32].

CONCLUSIONS

In conclusion, amlodipine has an impact on endogenous sulfur metabolism in different mouse systems, what is reflected by the free H_2S tissue concentrations changes in different mouse organs.

ACKNOWLEDGEMENTS

The study was supported by the grant No. K/ZBW/000175 from the Jagiellonian University Medical College.

Bogdan Wiliński[1], Jerzy Wiliński[2], Eugeniusz Somogyi[3], Joanna Piotrowska[3], Marta Góralska[1]

AMLODYPINA WYWIERA WPŁYW NA TKANKOWE STĘŻENIE SIARKOWODORU W RÓŻNYCH NARZĄDACH MYSZY

Streszczenie

Interakcje pomiędzy działaniem blokerów kanału wapniowego a endogennym siarkowodorem (H_2S) nie są znane. Myszom szczepu CBA podawano dootrzewnowo wodny roztwór zawierający amlodypinę w ilości 3 mg/kg masy ciała lub 10 mg/kg masy ciała dziennie. Grupa kontrolna otrzymywała sól fizjologiczną. Pomiary tkankowego stężenia wolnego siarkowodoru wykonywano przy użyciu zmodyfikowanej spektrofotometrycznej metody Siegela. Obserwowano spadek poziomu H_2S w mózgu i wątrobie w obydwu grupach. Niższa dawka amlodypiny powodowała wzrost stężenia H_2S w sercu i nerkach, podczas gdy wyższa znacząco obniżała tkankowy poziom H_2S w tych narządach. Wyniki naszego doświadczenia pokazują, że amlodypina ma wpływ na biologię endogennego siarkowodoru i powoduje zmiany w jego tkankowej koncentracji w różnych narządach myszy.

REFERENCES

1. *Kimura H.*: Hydrogen sulfide: its production, release and functions. Amino Acids 2011, 41: 113–121. — **2.** *Predmore B.L. Lefer D.J.*: Development of hydrogen sulfide-based therapeutics for cardiovascular disease. J. Cardiovasc. Transl. Res. 2010, 3: 487–498. — **3.** *Mason R.P., Marche P., Hintze T.H.*: Novel vascular biology of third-generation L-type calcium channel antagonists: ancillary actions of amlodipine. Arterioscler Thromb Vasc Biol 2003, 23: 2155–2163. — **4.** *Siegel L.M.*: A Direct Microdetermination for Sulfide. Anal Biochem 1965, 11: 126–132. — **5.** *Somogyi E., Piotrowska J., Rzeszutko W.*: An effect of some parameters of the determination of the hydrogen sulfide in pig liver and brain on the obtained results. Farm. Prz. Nauk. 2008, 5: 33–36. — **6.** *Phillips J.E., Preston Mason R.*: Inhibition of oxidized LDL aggregation with the calcium channel blocker amlodipine: role of electrostatic interactions. Atherosclerosis 2003, 168: 239–244. — **7.** *Xu B., Xiao-hong L., Lin G., Queen L., Ferro A.*: Amlodipine, but not verapamil or nifedipine, dilates rabbit femoral artery largely through a nitric oxide- and kinin-dependent mechanism. Br. J. Pharmacol. 2002, 136: 375–382. — **8.** *Gourine A.V., Pernow J., Poputnikov D.M., Sjoquist P.O.*: Calcium antagonist clevidipine reduces myocardial reperfusion injury by a mechanism related to bradykinin and nitric oxide. J Cardiovasc Pharmacol 2002, 40: 564–570. — **9.** *Stepien O., Zhang Y., Zhu D., Marche P.*: Dual mechanism of action of amlodipine in human vascular smooth muscle cells. J. Hypertens 2002, 20: 95–102. — **10.** *Ko Y., Totzke G., Graack G.H., et al.*: Action of dihydropyridine calcium antagonists on early growth response gene expression and cell growth in vascular smooth muscle cells. J. Hypertens. 1993, 11: 1171–1178.

11. *Mason R.P., Walter M.F., Trumbore M.W., Olmstead E.G., Jr. Mason P.E.*: Membrane antioxidant effects of the charged dihydropyridine calcium antagonist amlodipine. J. Mol. Cell. Cardiol. 1999, 31: 275–281. — **12.** *Yang C.M., Chien C.S., Hsiao L.D., et al.*: Mitogenic effect of oxidized low-density lipoprotein on vascular smooth muscle cells mediated by activation of Ras/Raf/MEK/MAPK pathway. Br. J. Pharmacol. 2001, 132: 1531–1541. — **13.** *Yue H., Uzui H., Shimizu H., et al.*: Different effects of calcium channel blockers on matrix metalloproteinase-2 expression in cultured rat cardiac fibroblasts. J. Cardiovasc. Pharmacol. 2004, 44: 223–230. — **14.** *Shibuya N., Tanaka M., Yoshida M., et al.*: 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. Antioxid. Redox. Signal. 2009, 11: 703–714. — **15.** *Ishigami M., Hiraki K., Umemura K., Ogasawara Y., Ishii K., Kimura H.*: A source of hydrogen sulfide and a mechanism of its release in the brain. Antioxid. Redox. Signal. 2009, 11: 205–214. — **16.** *Gadalla M.M., Snyder S.H.*: Hydrogen sulfide as a gasotransmitter. J Neurochem 2010, 113: 14–26. — **17.** *Łowicka E., Bełtowski J.*: Hydrogen sulfide (H2S) — the third gas of interest for pharmacologists. Pharmacol. Rep. 2007, 59: 4–24. — **18.** *Jeong S.O., Pae H.O., Oh G.S., et al.*: Hydrogen sulfide potentiates interleukin-1beta-induced nitric oxide production via enhancement of extracellular signal-regulated kinase activation in rat vascular smooth muscle cells. Biochem. Biophys. Res. Commun. 2006, 345: 938–944. — **19.** *Li L., Moore P.K.*: An overview of the biological significance of endogenous gases: new roles for old molecules. Biochem. Soc. Trans. 2007, 35: 1138–1141. — **20.** *Sun Y.G., Cao Y.X., Wang W.W., Ma S.F., Yao T., Zhu Y.C.*: Hydrogen sulphide is an inhibitor of L-type calcium channels and mechanical contraction in rat cardiomyocytes. Cardiovasc. Res. 2008, 79: 632–641.

21. *Hu Y., Chen X., Pan T.T., et al.*: Cardioprotection induced by hydrogen sulfide preconditioning involves activation of ERK and PI3K/Akt pathways. Pflugers. Arch. 2008, 455: 607–616. — **22.** *Calvert J.W., Jha S., Gundewar S., et al.*: Hydrogen sulfide mediates cardioprotection through Nrf2 signaling. Circ. Res. 2009, 105: 365–374. — **23.** *Sivarajah A., Collino M., Yasin M., et al.*: Anti-apoptotic and anti-inflammatory effects of hydrogen sulfide in a rat model of regional myocardial I/R. Shock 2009, 31: 267–274. — **24.** *Sodha N.R., Clements R.T., Feng J., et al.*: Hydrogen sulfide therapy attenuates the inflammatory response in a porcine model of myocardial ischemia/reperfusion injury. J. Thorac. Cardiovasc. Surg. 2009, 138: 977–984.

— 25. *Oh G.S.*, *Pae H.O.*, *Lee B.S.*, *et al.*: Hydrogen sulfide inhibits nitric oxide production and nuclear factor-kappaB via heme oxygenase-1 expression in RAW264.7 macrophages stimulated with lipopolysaccharide. Free Radic. Biol. Med. 2006, 41: 106–119. **— 26.** *Sen U.*, *Basu P.*, *Abe O.A.*, *et al.*: Hydrogen sulfide ameliorates hyperhomocysteinemia-associated chronic renal failure. Am. J. Physiol. Renal. Physiol. 2009, 297: F410–409. **— 27.** *Li L.*, *Hsu A.*, *Moore P.K.*: Actions and interactions of nitric oxide, carbon monoxide and hydrogen sulphide in the cardiovascular system and in inflammation — a tale of three gases! Pharmacol. Ther. 2009, 123: 386–400. **— 28.** *Srebro Z.*, *Somogyi E.*, *Wiliński B.*, *Góralska M.*, *Wiliński J.*, *Sura P.*: Aspirin augments the concentration of endogenous hydrogen sulfide in mouse brain and liver. Folia Med. Cracov. 2006, 47: 87–91. **— 29.** *Wiliński J.*, *Somogyi E.*, *Góralska M.*, *Wiliński B.*, *Czarnecka D.*: Ramipril enhances the endogenous hydrogen sulfide tissue concentration in mouse heart and brain. Folia Med. Cracov. 2008, 3–4: 123–130. **— 30.** *Wiliński B.*, *Wiliński J.*, *Somogyi E.*, *Góralska M.*, *Piotrowska J.*: Ramipril affects hydrogen sulfide generation in mouse liver and kidney. Folia Biol. (Kraków) 2010, 3–4: 177–180.

31. *Wiliński B.*, *Wiliński J.*, *Somogyi E.*, *Piotrowska J.*, *Góralska M.*: Atorvastatin affects the hydrogen sulfide tissue concentration in mouse kidneys and other organs. Pharmacol. Rep. 2011, 63: 184–188. **— 32.** *Wiliński B.*, *Wiliński J.*, *Somogyi E.*, *Góralska M.*, *Piotrowska J.*: Paracetamol (acetaminophen) decreases hydrogen sulfide tissue concentration in brain and increases in the heart, liver and kidney in mice. Folia Biol. (Kraków) 2011, 59: 41–44.

[1] Zakład Biologii Rozwoju Człowieka
Wydział Nauk o Zdrowiu
Uniwersytet Jagielloński Collegium Medicum
ul. Kopernika 7, 31-034 Kraków, Poland
Phone: +48 12 422 99 49

[2] I Klinika Kardiologii i Nadciśnienia Tętniczego
Uniwersytet Jagielloński Collegium Medicum
ul. Kopernika 17, 31-501 Kraków, Poland
Phone: +48 12 424 73 00

[3] Katedra Chemii Nieorganicznej i Analitycznej
Uniwersytet Jagielloński Collegium Medicum
ul. Medyczna 9, 30-688 Kraków, Poland
Phone: +48 12 657 04 80

Address for correspondence:
Bogdan Wiliński MD, PhD
Department of Human Developmental Biology
Jagiellonian University Medical College
ul. Kopernika 7, 31-034 Kraków, Poland
Phone: +48 12 422 99 49
e-mail: bowil@interia.pl