Carvedilol Induces Endogenous Hydrogen Sulfide Tissue Concentration Changes in Various Mouse Organs*

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Accepted May 19, 2011

Carvedilol, a third generation non-selective adrenergic receptor blocker, is widely used in cardiology. Its action has been proven to reach beyond adrenergic antagonism and involves multiple biological mechanisms. The interaction between carvedilol and endogenous 'gasotransmitter' hydrogen sulfide (H$_2$S) is unknown. The aim of the study is to assess the influence of carvedilol on the H$_2$S tissue level in mouse brain, liver, heart and kidney. Twenty eight SJL strain female mice were administered intraperitoneal injections of 2.5 mg/kg b.w.d. (group D1, n = 7), 5 mg/kg b.w.d. (group D2, n = 7) or 10 mg/kg b.w.d. of carvedilol (group D3, n = 7). The control group (n = 7) received physiological saline in portions of the same volume (0.2 ml). Measurements of the free tissue H$_2$S concentrations were performed according to the modified method of Siegel. A progressive decline in H$_2$S tissue concentration along with an increase in carvedilol dose was observed in the brain (12.5%, 13.7% and 19.6%, respectively). Only the highest carvedilol dose induced a change in H$_2$S tissue level in the heart – an increase by 75.5%. In the liver medium and high doses of carvedilol increased the H$_2$S level by 48.1% and 11.8%, respectively. In the kidney, group D2 showed a significant decrease of H$_2$S tissue level (22.5%), while in the D3 group the H$_2$S concentration increased by 12.9%. Our study has proven that carvedilol affects H$_2$S tissue concentration in different mouse organs.

Key words: Hydrogen sulfide, carvedilol, adrenergic beta-antagonists, nitric oxide, mice.

Carvedilol, a third generation non-selective adrenergic receptor blocker, is widely used in cardiology and general practice in the treatment of chronic diseases like congestive heart failure and arterial hypertension (CHAKRABORTY et al. 2010). The action of carvedilol has been proven to reach beyond adrenergic antagonism and comprises i.a. antioxidant activity, calcium channel blockade and nitric oxide (NO) production enhancement (KOSTKA-JEZIERNY & TYKARSKI 2009). On the other hand, endogenously formed 'gasotransmitter' hydrogen sulfide (H$_2$S) has been identified as a crucial regulator of circulatory, nervous, gastrointestinal and excretory systems. Altered production of H$_2$S was observed in arterial hypertension, myocardial ischemia and atherosclerosis (ŁOWICKA & BELTOWSKI 2007). The interaction between carvedilol and endogenous H$_2$S is unknown.

The aim of the study is to assess the influence of carvedilol on the endogenous tissue H$_2$S concentrations in mouse brain, heart, liver and kidney.

Material and Methods

Animals

Twenty eight SJL strain female mice (11-12 week old individuals) of approximately 20 g weight were involved in the study. The animals were housed under standard laboratory conditions and had free

*Supported by the grant No. K/ZBW/000175 from the Jagiellonian University Medical College.
access to water and food. They were kept at a temperature of 22-24°C with a light/dark cycle of 12 h.

Study protocol

A non-selective lipophilic β-blocker / α-1 blocker carvedilol (Avedol, Polpharma, Poland) was dissolved in physiological saline. The study design comprised intraperitoneal injections of 2.5 mg per kg of body weight of carvedilol daily (group D1, n = 7), 5 mg per kg of body weight of carvedilol daily (group D2, n = 7) or 10 mg per kg of body weight of carvedilol daily (group D3, n = 7) for 5 consecutive days at the same time of day (10:30 am), each administration consisted of 0.2 ml of the solution. The control population (n = 7) 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 carvedilol well and remained in good condition till the end of the experiment. Measurements of the free tissue H$_2$S concentration were performed by the use of the modified method of Siegel (SIEGEL 1965; SOMOGYI et al. 2008). The study has been performed in accordance with the guidelines for the care and use of laboratory animals accepted by Bioethical Committee of the Jagiellonian University Medical College (Kraków, Poland).

Tissue sample preparation

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): brain tissue in proportion of 1 to 4, liver and kidney of 1 to 5 and 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 brain or liver and 0.75 ml heart or kidney supernatant samples 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.

H$_2$S tissue concentration measurements

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$_2$S) solution. For each group of animals four concurrent analyses of each analyzed tissue type were performed.

Statistical analysis

Statistical analysis was performed within the R Environment by the Student’s t-test and univariate analysis of variance (ANOVA). Statistical significance was considered when P<0.05.

Results and Discussion

Progressive H$_2$S tissue concentration decline was observed in the brain along with a rising carvedilol dose (by 12.5%, 13.7% and 19.6%, respectively). In the heart only the highest carvedilol dose induced H$_2$S tissue level change, but the increase was spectacular, reaching 75.5%. In the liver, medium and high doses altered the H$_2$S concentration – 5 mg/kg b.w./d of carvedilol increased the H$_2$S level by 48.1% and 10 mg/kg b.w/d by 11.8%. In the kidney, the group D2 showed a significant decrease of H$_2$S tissue level (22.5%), while in the D3 group H$_2$S concentration increased by 12.9%. In the variance analysis for each tissue type only H$_2$S concentration changes within the heart and brain were statistically significant (Table 1). Noteworthy, each organ has different metabolism, paracrine and endocrine regulation, and specific transmitter interactions, thus variable changes of H$_2$S concentrations only confirm this complexity and heterogeneity.

Carvedilol’s therapeutic actions could not be fully explained by adrenoreceptor blockade. Numerous studies have provided evidence that carvedilol has various other properties including antioxidant action, calcium channel antagonism, anti-inflammatory actions: fall in interleukin-1 (IL-1), interleukin-6 (IL-6), c-reactive protein (CRP) and tumor necrosis factor-α (TNF-α), direct inhibition of transcription factors like NF-κB, low density lipoproteins (LDL) oxidation; stabilization of atherosclerotic plaques by decreasing intercellular adhesion molecule-1 (ICAM-1) and activity of matrix metalloproteinases 2 and 9 (MMP-2, MMP-9); prevention of endothelial and myocardium apoptosis, reversal of cardiac remodeling in chronic heart failure and endothelin-1 (ET-1) suppression (BELLENGER et al. 2004; KALINOWSKI et al. 2003; KOSTKA-JEZIERNY & TYKARSKI 2009; ROMEO et al. 2000; RUFFOLO et al. 1993). Moreover, the major proportion of carvedilol’s biological action, especially regarding hypotensive and vascular effects, seems to be mediated by NO, whose level rises due to endothelial nitric oxide synthase (NOS) stimulation (AFONSO et al. 2006).
Hydrogen sulfide (H$_2$S) is endogenously formed from L-cysteine in several enzymatic reactions catalyzed by cystathionine $\beta$-synthase (CBS), cystathionine $\gamma$-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3MST), and in non-enzymatic pathways in many tissues. Cytoplasmatic bound sulfur is postulated to absorb and store exogenously applied and endogenously produced H$_2$S which is released from the bound sulfur pool in the presence of physiologic concentrations of glutathione and cysteine in slightly alkaline conditions (ISHIGAMI et al. 2009). H$_2$S acts as a ‘gasotransmitter’ and serves as a co-modulator of various physiological and pathophysiological processes such as regulation of vascular tone, myocardial contractility, neurotransmission and perception (FIORUCCI et al. 2006; SHIBUYA et al. 2009). Its biological action comprises numerous intracellular mechanisms including adenosine triphosphate (ATP)-sensitive potassium channels (K$_{ATP}$) stimulation, sulphydrylation of different proteins and maintaining protein–SH groups in the reduced state, reaction with reactive oxygen and nitrogen species (ROS and RNS) (ŁÓWICKA & BELTOWSKI 2007; SUN et al. 2008). H$_2$S interacts with carbon monoxide (CO) and nitric oxide (NO) in a number of ways including affecting each other’s synthesis and biological responses within target tissues. All these three gases bind to haemoglobin and impede mitochondrial oxidative phosphorylation by inhibiting cytochrome c oxidase (LI et al. 2009). Analogically to carvedilol, H$_2$S decreases IL-6, TNF-$\alpha$ levels, reduces the activation of NF-$\kappa$B complex and the activity of MMP-2 and MMP-9 (OH et al. 2006; SEN et al. 2009; SODHA et al. 2009).

Our study has shown that carvedilol’s action involves H$_2$S, probably via its production rate alteration and release with possible NO share as one of the mechanisms. Some of the biological effects of the beta-blocker and H$_2$S are common. It is unknown whether H$_2$S mediates any of them and to what extent carvedilol’s biology is dependent on the messenger, since research dedicated to the issue has not been done. In the heart, the cardioprotective effects of H$_2$S have been demonstrated to involve opening of K$_{ATP}$ channels as well as effects of preserving mitochondrial structure and function (ELROD et al. 2007). It might be an accessory effect to NO beneficial impact on intracellular transcription factors resulting in increased expression of cardioprotective proteins like superoxide dismutase (SOD), inducible NOS (iNOS), cyclooxygenase-2 (COX-2) and heat shock proteins (DAWN & BOLL1 2002). In hepatology, carvedilol appears to be a potentially viable option for treating portal hypertension along with angiotensin-converting enzyme inhibitors (ACEI) (HEMSTREET 2004). H$_2$S regulates perfusion pressure in normal and cirrhotic liver in a NO-independent manner; NO and H$_2$S are released by different cellular sources and their hemodynamic effects involve different cellular targets. H$_2$S generation in cirrhosis is decreased due to a reduced expression/activity of CSE in hepatic stellate cells (HSC) — one of the main sources of H$_2$S in the liver (FIORUCCI et al. 2005). In our previous studies a tissue specific ACEI ramipril also enhanced the H$_2$S tissue level in the liver, heart and kidney (WILINSKI et al. 2010; WILINSKI et al. 2008). In kidneys H$_2$S has been recognized as an important regulator of renal function affecting both vascular and tubular actions (XIA et al. 2009). Some studies with carvedilol demonstrate atten-
aged increases in albuminuria as well as reduction in cardiovascular events in chronic kidney disease patients with hypertension (BAKRIS et al. 2006). Carvedilol also protected against the renal mitochondrial toxicity induced by cisplatin and daunorubicin-induced cardiotoxicity and nephrotoxicity in rats (AROZAL et al. 2010; RODRIGUES et al. 2010).

H₂S acts as a neuromodulator as well as an intracellular messenger in the central nervous system. Its perturbed metabolism has been investigated in many neurological disorders including Alzheimer’s disease (QU et al. 2008). Recently, carvedilol was found to improve neuronal transmission and attenuate brain oligomic bet-amyloid content and cognitive deterioration in two mouse models of Alzheimer’s disease (WANG et al. 2010). Analogically, non-steroidal anti-inflammatory drugs were also demonstrated to exert some anti-amyloidogenic effects and to affect H₂S biology (BILSKA et al. 2010; SREBRO et al. 2006).

In conclusion, carvedilol affects the H₂S tissue concentrations in mouse brain, heart, liver and kidney. The involvement of H₂S makes the biological action of carvedilol more complex and opens new fields for investigation for both of them in neurology, cardiology, hepatology and nephrology. Several biotechnology companies are already developing H₂S-based therapeutic compounds, and there are ongoing clinical trials investigating the therapeutic potential of H₂S (PREDMORE & LEFER 2010).

References


Carvedilol and the Endogenous Hydrogen Sulfide


